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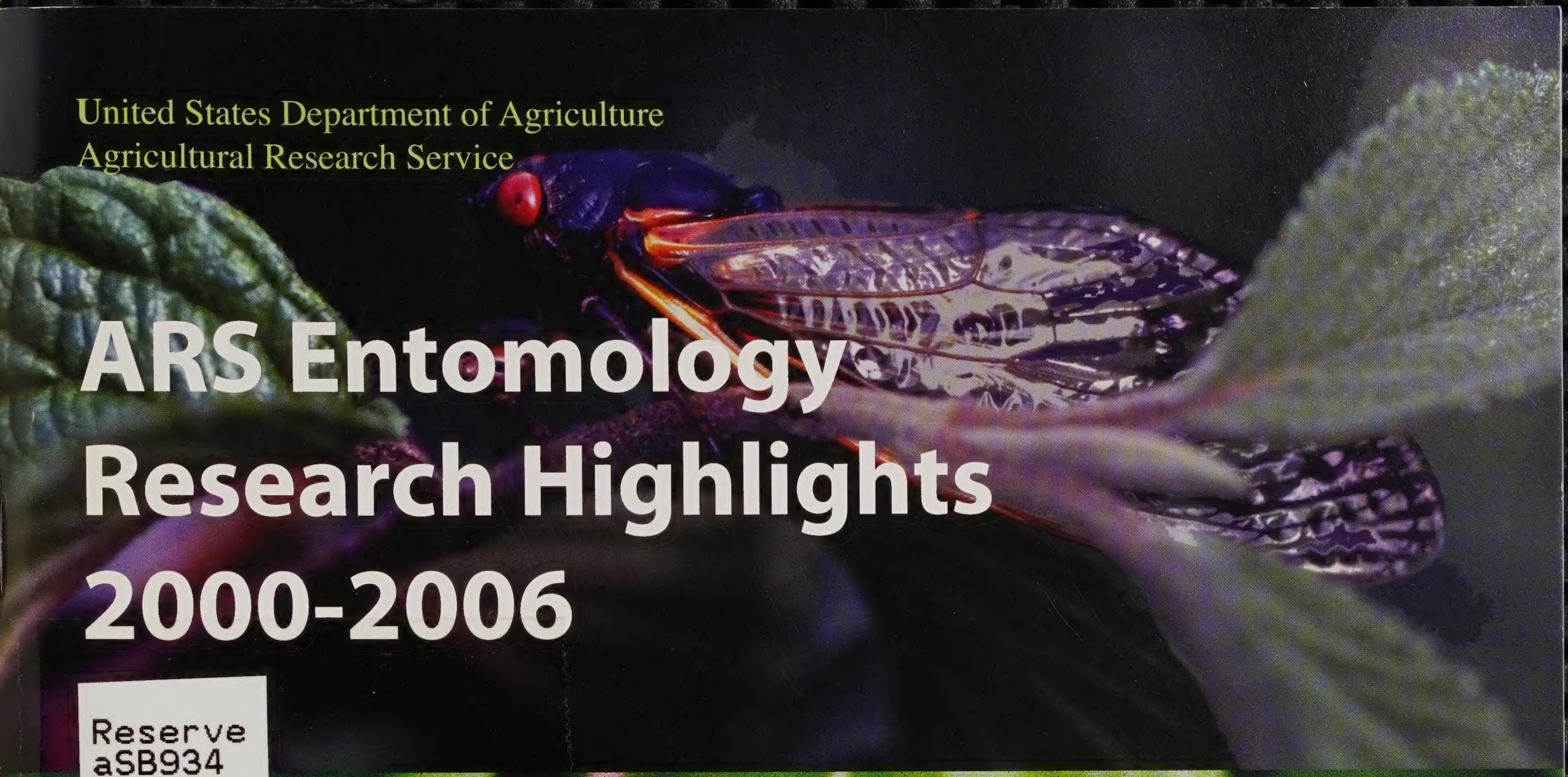

ARS Entomology Research Highlights 2000-2006

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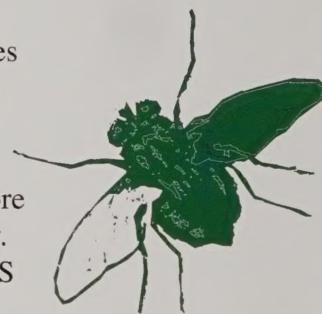
Foreword

This *Agricultural Research* magazine reprint volume contains selected entomology research articles previously published in the *Agricultural Research* magazine between January 2000 and December 2006. The assistance of the Agricultural Research Service (ARS) Information Staff in providing the excellent articles and photographs and their assistance in publishing this volume is greatly appreciated.



For additional information concerning ARS programs and the more than 1,200 research projects carried out by ARS, visit http://www.ars.usda.gov/research/program.htm or contact one of the four ARS national program leaders responsible for entomology programs: Kevin Hackett (301) 504-4680, Ernest Delfosse (301) 504-6918, Kenneth Vick (301) 504-5321, Dan Strickman (301) 504-5771.

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This volume contains selected entomology research articles reprinted from *Agricultural Research* magazine. The articles were published between January 2000 and December 2006. The magazine is published by the U.S. Department of Agriculture, Agricultural Research Service, 5601 Sunnyside Ave., Beltsville, MD 20705-5130; phone (301) 504-1651; fax (301) 504-1641. The magazine is available on the World Wide Web at ars.usda.gov/ar.

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Issued November 2007

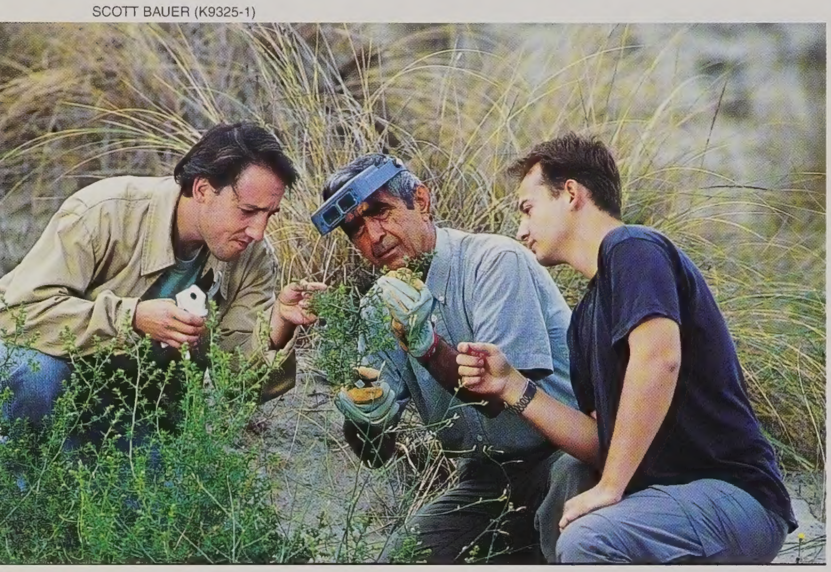
Entomology and Other Career Opportunities in ARS

Agricultural Research Service (ARS) is the principal in-house research agency of the United States Department of Agriculture (USDA). As the largest agricultural research organization in the world, ARS employs over 8,000 people, more than 2,000 of whom are scientists.

ARS has over 100 research facilities strategically located in major farm and rangeland ecosystems throughout the United States and some foreign countries. Many locations are on or near major college and university campuses.

PEGGY GREB (K11160-3)

SCOTT BAUER (K9325-1)



The agency employs research entomologists at approximately 36 locations across the continental United States and at Fairbanks, Alaska; Hilo, Hawaii; Montpellier, France; and Panama City, Panama (see map on back cover to view locations).

ARS is one of the largest, if not the largest, employers of research entomologists in the world. In the agency, about 280 entomologists conduct research on various topics—about 100 on crop pest management, 60 on basic research (including systematics), 60 on biological control and honey bees, 40 on insects of medical and veterinary importance, and 20 on fruitflies, quarantine treatments, and methyl bromide replacement. Many other ARS entomologists serve in research support positions, and several work in executive positions.

PEGGY GREB (K10225-1)

ARS offers the most diverse opportunities for entomologists of any organization in the world. The agency's research is broad in scope and includes insect pests of crops (for example, cotton, wheat, tree fruit and nuts, and vegetables), insect pests of medical and veterinary importance, insect pests of stored products, biological control, integrated pest management, insect pests of quarantine importance, and insect pests of urban significance. ARS entomologists are also involved in basic research on insect biology, physiology, ecology, systematics, and attractants. As varied as these topics are, they are still just a few

JACK DYKINGA (K7637-7)

PEGGY GREB (K10222-1)

PEGGY GREB (K9645-1)

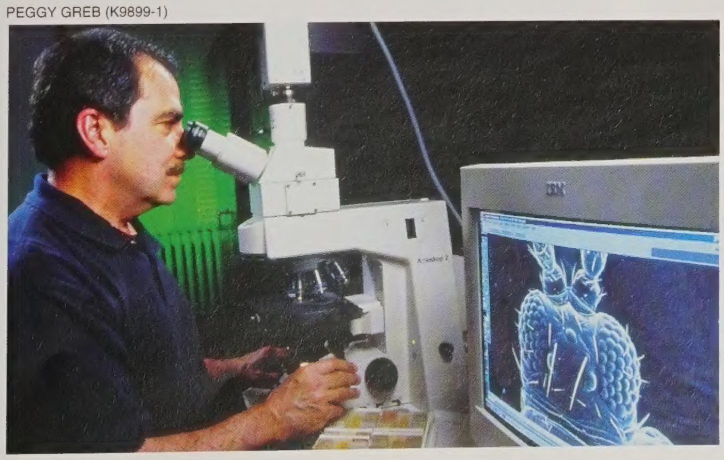
of the many examples of the wide range of ARS's entomology research program.

ARS entomologists also provide research support for other federal and state agencies. These responsibilities include research support of USDA's Animal and Plant Health Inspection Service, including programs on eradicating screwworm, boll weevil, and fruit fly; developing and evaluating quarantine treatments to expand U.S. markets for agricultural products; protecting American agriculture from exotic pests; providing expert identification of insects; and developing insect repellents and other pest protection measures for the military. ARS research conducted on biological control of weeds is used by several different federal and state agencies. Many people in private industry (for example, chemical companies, aerial applicators, companies marketing traps and attractants, and private consultants) benefit from technology developed by ARS entomologists.

STEPHEN AUSMUS (K10285-1)

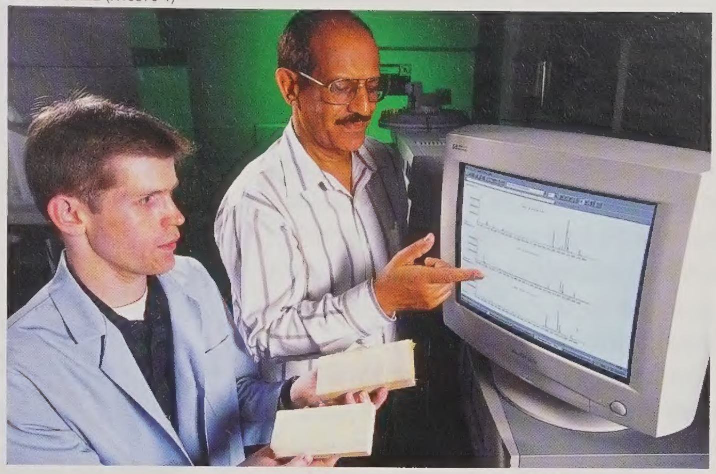
Many entomologists in ARS are research scientists with advanced degrees, but abundant career opportunities also exist for undergraduate entomologists. Entomologists who serve as research technicians and support scientists are a critical part of the research programs and have ample opportunity for advancement. And, because many of our research locations are on or near colleges, our entomologists have opportunities to enhance their training and also pursue advanced degrees.

PEGGY GREB (K9899-1)



The career opportunities ARS offers are numerous and challenging. Besides

PEGGY GREB (K10570-1)



entomology, ARS research covers agricultural engineering, agronomy, animal science, biochemistry, biology, chemistry, food safety, genetics, hydrology, microbiology, nutrition, soil science, veterinary science, and virology. The agency employs specialists, technicians, and assistants in occupational areas such as administrative management, budget and accounting, human resources, information technology, library services, and procurement and contracting. Temporary seasonal and student workers are also hired at most locations.

SCOTT BAUER (K8901-1)

ARS is an outstanding organization in which to pursue a career in agricultural research. Promotions for scientists are based on the impact of the individual research accomplished. Most of the research facilities are modern, and the scientific equipment is usually state-of-the-art. Our scientists are encouraged to seek out cooperative research projects with other ARS scientists, as well as with scientists from colleges, universities, experiment stations, and private industry.

To pursue a possible career with ARS, visit our careers web page at http://www.ars.usda.gov/careers.—By **James R. Coppedge, Thomas J. Henneberry,** and **Larry D. Chandler.**

For Males Only

Temperature-Sensitive Medflies

SCOTT BAUER (K8899-1

Geneticist Don McInnis collects a pair of mating medflies in an outdoor field cage during a mating competitiveness test.

There are millions of these six-legged bachelors in sunshine states like California and Florida. Because they're small and unobtrusive, you're unlikely to ever notice them. But their success in the game of love helps protect your favorite fruits and vegetables from becoming an icky, maggoty mess.

What are they?

Temperature-sensitive lethal Mediterranean fruit flies—or TSLs for short. Through a bit of laboratory trickery, TSL medflies that work outdoors are exclusively males. All of them are sterile—that is, infertile. Dropped from airplanes to work in areas where invading medflies have been detected, the TSL medflies have an important assignment: Find and mate with wild female Mediterranean fruit flies.

These fertile females would like to make a home for their offspring in warm-weather states. When they mate with sterile male medflies, however, no viable offspring are produced. Deprived of new generations of healthy young flies, the population soon crashes.

Now, 6 years of tests by USDA scientists and their colleagues in lush coffee plantations of southwestern Guatemala have shown that what's known as the Toliman strain of TSLs do their job anywhere from three to five times better than conventional, mixed-sex strains.

Temperature-sensitive lethal medflies get their name from the fact that high temperatures can be lethal to eggs containing TSL females. This genetic quirk in females was discovered by researchers working with the International Atomic Energy Agency in Vienna, Austria, about a decade ago.

The TSL trait allows mass-rearing of medflies that are exclusively males. And on a per-male basis, TSL males don't cost any more to rear than standard, mixed-sex strains of medflies. That saves resources that would otherwise go into producing the unneeded females. What's more, not having sterile females

SCOTT BAUER (K8902-1)

Male (brown) and female (white) med-fly pupae. Pupae color was the basis of the old method of separating males from females.

to distract them once they are outdoors and looking for wild, fertile females may be a key to the TSL males' success.

To produce TSL males requires insectary workers to bathe the white, banana-shaped medfly eggs in 97 °F water for 12 to 24 hours. That kills all the eggs with female embryos inside but doesn't harm those with males. Later, when they are pupae—their final developmental stage before becoming adult flies—the insects are irradiated for several minutes to render them sexually sterile.

Using temperature-sensitive lethal medflies, says ARS research geneticist Donald O. McInnis, is an effective, environmentally friendly strategy that helps reduce the need for chemical insecticides such as malathion. Medfly, *Ceratitis capitata,* is one of the world's worst insect pests of agriculture. It can infest more than 250 different kinds of fruits and vegetables and easily cost millions of dollars to eradicate. If it were to become established in California, for example, this industrious, one-third-inch-long insect could make a dent of anywhere from $324 to $510 million in the state's economy every year.

SCOTT BAUER (K8898-2)

Male medfly resting on a leaf.

If medflies were to become established in California, this industrious, one-third-inch-long insect could make a dent of anywhere from $324 to $510 million in the state's economy every year.

Medflies ruin crops when fertile females use their tubelike ovipositors to punch holes in the skin of a ripening fruit or vegetable, then pump their eggs inside. The eggs hatch and produce wiggly medfly larvae that develop inside the fruit, feeding on the flesh and making it unmarketable. They eventually leave the fruit, dropping to the ground to continue their development into adult flies that begin the cycle all over again.

McInnis, who is at ARS' U.S. Pacific Basin Agricultural Research Center in Honolulu, Hawaii, helped design the pioneering tests of the Toliman TSL medflies in Guatemala. He did the work with David Lance of USDA's Animal and Plant Health Inspection Service, formerly at Waimanalo, Hawaii, and now at Cape Cod, Massachusetts; Pedro Rendon and colleagues with APHIS in Guatemala City, Guatemala; and co-researchers from the government of Guatemala.

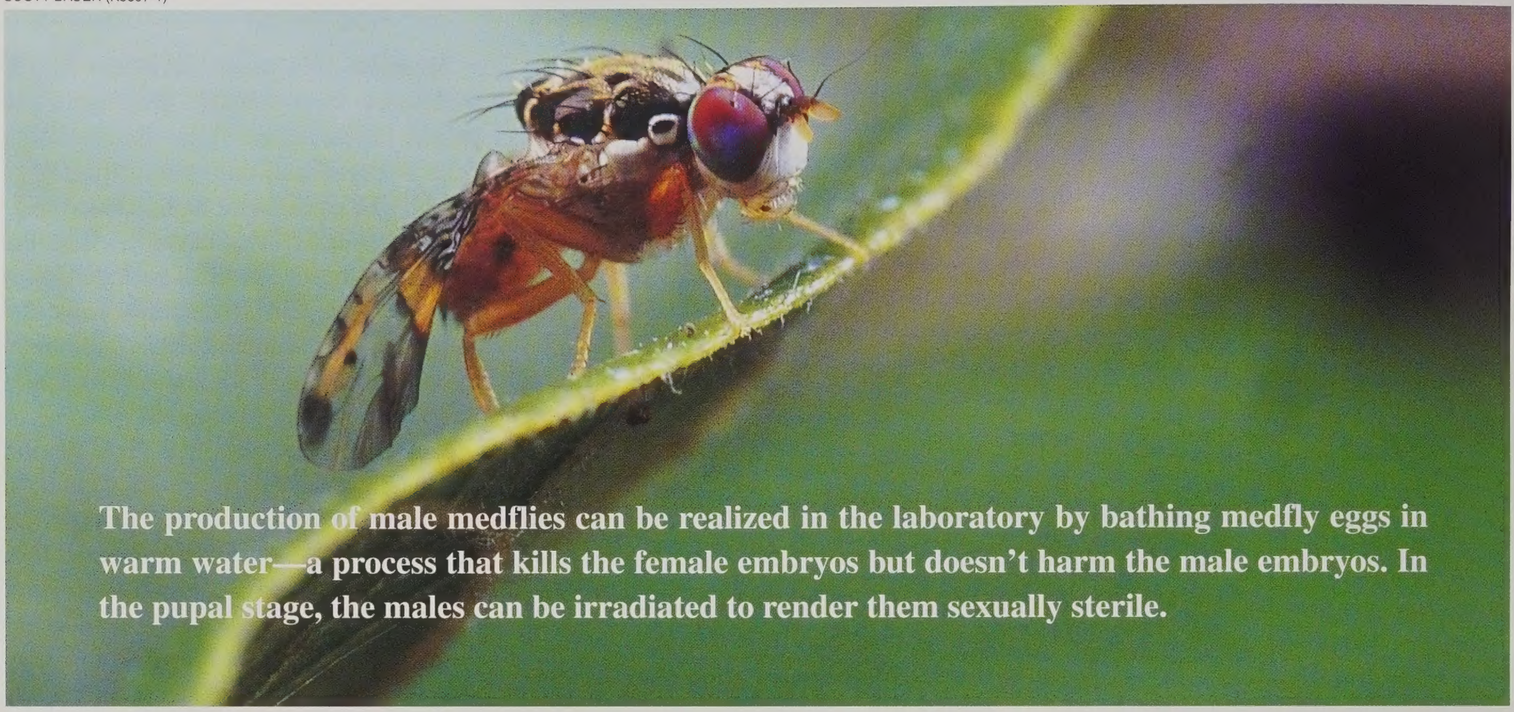
Rendon and staffers at the International Atomic Energy Agency developed the Toliman TSL strain by crossing the Viennese TSL flies with a Guatemalan strain of medfly. Toliman TSLs needed for the collaborative research were produced at a medfly factory in El Pino, outside Guatemala City, Guatemala. APHIS and the Guatemalan government run that insectary.

Native to Africa, medflies have lived in Guatemala since at least the 1960s. The El Pino factory can produce up to about 50 billion medflies a year. Some are released in Guatemala, forming a living barrier that helps stop the species from advancing north into the United States. Other steriles are shipped to the United States for use in regional wars against medfly.

California, for instance, has an enormous need for steriles for its ongoing campaign in Los Angeles, Riverside, Orange, and San Bernardino counties.

The state last year used 15 billion steriles for this war on medflies. Florida has a similar campaign and uses 70 million

SCOTT BAUER (K8897-1)



The production of male medflies can be realized in the laboratory by bathing medfly eggs in warm water—a process that kills the female embryos but doesn't harm the male embryos. In the pupal stage, the males can be irradiated to render them sexually sterile.

sterile medflies a week in a 500-square-mile area.

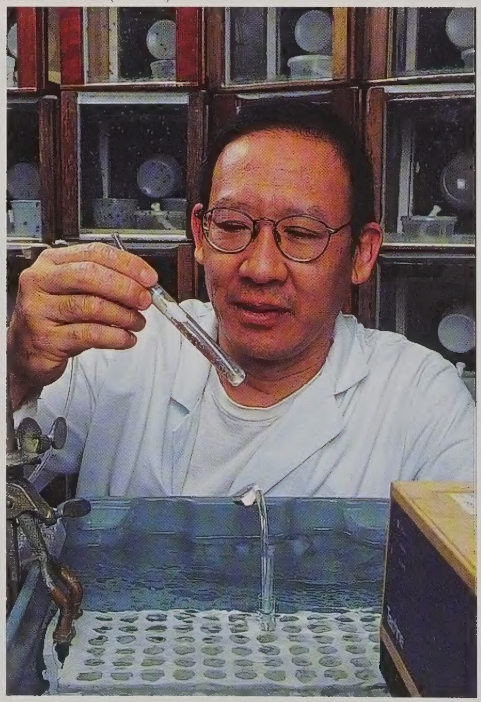
The Guatemalan studies, begun in 1993, included the largest-ever outdoor test of TSL medflies. For these rigorous examinations of the insects' proficiencies, more than 120 million Toliman TSLs and the lab's conventional "Petapa" strain of neutered medflies were set free in coffee fields.

As part of the experiment, researchers collected coffee berries that had been visited by the female flies to determine whether the hidden eggs were infertile. In plots where the Toliman TSLs had been released, the proportion of infertile eggs to fertile eggs was from three to five times greater than that of a standard lab-reared strain.

The Toliman TSLs also scored high in two other critical categories: field dispersal, or ability to roam throughout fields instead of staying in one place, and field survival, or ability to adapt to climate and other environmental conditions.

The ARS, APHIS, and Guatemalan investigators were the first to move TSLs out of the lab and into mass production. As a result of the team's exhaustive studies of Toliman TSLs, the El Pino fly factory is now planning to produce only this top-performing strain.

SCOTT BAUER (K8901-1)



Technician Steven Tam checks medfly eggs prior to heating them in a 97 °F water bath to kill the females.

Two major sterile-medfly factories in Hawaii—one operated by APHIS and the other by the State of California—also plan to produce TSLs exclusively. In all, they could provide more than 400 million steriles a week for mainland campaigns.

Largely because of the impressive results of the Guatemalan field tests with Toliman TSLs, other countries threatened by medfly invasions are opting to use TSLs in their own mass-rearing programs. Chile and the Portuguese island of Madeira, for example, are already using strains of top-performing TSLs to guard their crops against the ravages of wild medflies.—By **Marcia Wood,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at http://www.nps.ars.usda.gov /programs/cppvs.htm.

Donald O. McInnis is at the USDA-ARS U.S. Pacific Basin Agricultural Research Center, 2727 Woodlawn Dr., Honolulu, HI 96822; phone (808) 988-8232, fax (808) 988-7290, e-mail dmcinnis@pbarc.ars.usda.gov. ◆

Bait-and-Kill Strategy Can Slash Insecticide Use in Corn Belt

Field tests in the Corn Belt have proven that "aerial spraying with a bait slurry can drastically reduce the amount of insecticide active ingredient used," says ARS entomologist Laurence D. Chandler.

Chandler is describing a successful conclusion to the corn rootworm areawide integrated pest management (IPM) project. "It's in its last year, and next year we will complete the handover to IPM consultants who will help farmers adopt practices we developed in conjunction with university scientists," Chandler says. He's at the ARS Red River Valley Agricultural Research Center, Fargo, North Dakota.

Chandler says the bait testing began with the 1997 corn crop and was part of one of the first areawide projects to target pests of a row crop. Chandler is program coordinator for the project.

"During the program, we tested two new, low-insecticide baits that use either a watermelon juice ingredient or wild buffalo gourd root powder because both contain cucurbitacins that act as feeding stimulants to adult rootworm beetles. We also evaluated a trap that uses corn rootworm attractants derived from plant-produced chemicals," Chandler says.

"All these products came from technology that ARS helped develop and became commercially available during the areawide program. The product evaluations were done at four 16-square-mile sites across the Corn Belt—at the Indiana-Illinois border and in Iowa, Kansas, and South Dakota—and at smaller study sites in Texas."

The baits use doses of insecticide at rates of an ounce or less per acre—95 to 98 percent less active ingredient than in conventional sprays. They are sprayed aerially on corn leaves where the beetles eat. The bait lands on leaves and forms individual drops containing cucurbitacins and insecticide. The cucurbitacins cause the beetles to feed preferentially on the drops and ingest a lethal dose of insecticide.

KEN HAMMOND (K7802-1)

Entomologists Larry Chandler (left) and Wayne Buhler check a corn ear for corn rootworm damage.

"If used throughout the Corn Belt, the bait sprays could reduce total pesticide use on corn by half," Chandler says.

Corn rootworms are the target of almost half the insecticides used in row crops in this country, requiring more insecticide than any other pest. "About 25 million acres of corn are treated each year," Chandler says. "In some years, rootworms can cost farmers up to $1 billion in crop losses and spraying expenses."

The baits are sprayed only when IPM scouts or farmers find an average of one female beetle per plant or when populations in various traps exceed a certain threshold. That is the level at which they can begin to cause significant economic harm to a farmer, enough to justify the expense of spraying.

Chandler explains that it is the beetle's offspring—the larvae—that eat corn roots. But the larvae are harder to count than beetles because they are underground.

"Under standard methods, corn farmers apply soil insecticide as a preventive measure, even though it's only needed less than half of the time. By targeting the beetle parents, we attempt to keep larvae levels low for the next corn season," Chandler says.

The baits do not pose any risk to bees or other beneficial insects, such as ladybugs. And the bitter cucurbitacin doesn't appeal to any other insects, Chandler says. "The musky smell released when a cantaloupe is cut open comes primarily from cucurbitacin, which is also found in cucumbers and squash."—By **Don Comis,** ARS.

This research is part of Crop Protection and Quarantine (#304) and Integrated Agricultural Systems (#207), two ARS National Programs described on the World Wide Web at http://www.nps.ars.usda.gov.

Laurence D. Chandler is at the USDA-ARS Red River Valley Agricultural Research Center, 1307 N. 18th Street, Fargo, ND 58105-5677; phone (701) 239-1371, fax (701) 239-1395, e-mail chandlel@fargo.ars.usda.gov. ◆

Scientists in Montpellier are

Exploring the World for Biocontrol Agents

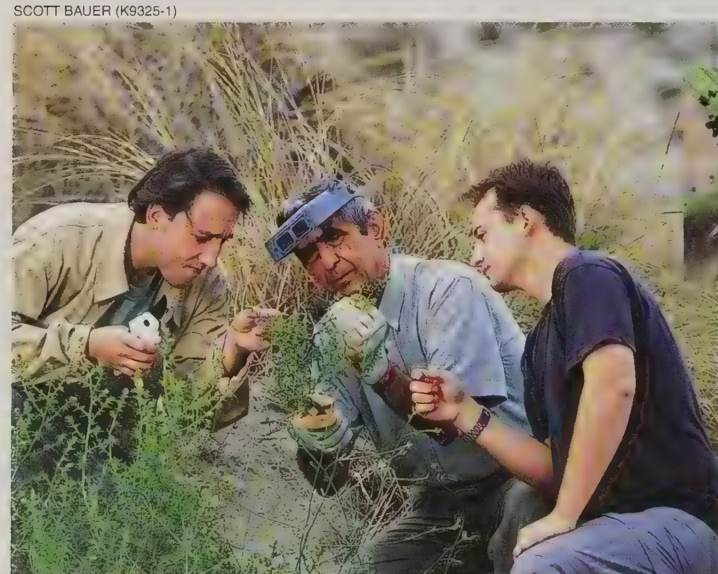
In a beautiful valley near Montpellier in southern France, the European Biological Control Laboratory is home to scientists who fight the battle against Eurasian weeds and insect pests that have invaded the United States.

SCOTT BAUER (K9341-2)

Seasoned travelers, the scientists at the European Biological Control Laboratory (EBCL) will happily oblige the curious with a story or two of misadventure. During a recent trip to Nepal, Tim Widmer learned first-hand just how tricky it can be to shake leeches off your pants and fingers. Rouhollah Sobhian tells of getting blinded in a Tunisian sandstorm, while Alan Kirk recalls being stranded in the Australian Outback. Then, there's Kim Hoelmer, who sampled fried grasshoppers in a Beijing street market, and Franck Hérard, whose team searched for a "lost" member in a thick forest only to find him waiting at their hotel.

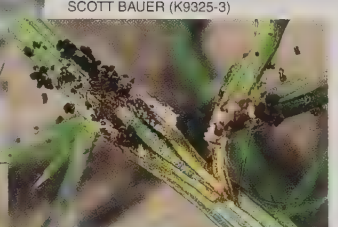
Listening to these scientists gives the impression that such misadventures are all part of the job. Aside from world travel, their duties include research on parasites, predators, and pathogens of insects and weeds that have become invasive pests of U.S. agriculture. By one estimate, these pests cost about $46 billion annually in losses and control, not counting ecological damage and harm to wildlife.

SCOTT BAUER (K9325-1)



In southern France, ARS specialist Rouhollah Sobhian (center) and technicians Arnaud Blanchet (left) and Boris Fumanal inspect Russian thistle for potential biocontrol insects.

SCOTT BAUER (K9325-3)



Well camouflaged, a larva of the *Gymnancylie canella* moth burrows into a Russian thistle stem.

From Montpellier's seaside airport, EBCL scientists routinely hop flights to the pests' points of origin in North Africa, the Middle East, the Balkans, and Asia to collect natural enemies. Typically, they'll explore sites where the crops, climate, or habitat matches a particular U.S. region where a pest has become established and a biocontrol agent is needed.

"With all the concern about invasive, nonnative species, we're on the forefront of the only proven, sustainable technology to deal long-term with the problems that have already been introduced," says EBCL director Paul C. (Chuck) Quimby, Jr.

EBCL is about a 20-minute drive from downtown Montpellier on France's Mediterranean coast. USDA's Agricultural Research Service (ARS) established the lab in 1919 and had a new facility built for it in 1999 on a 5-acre plot inside France's prestigious international research campus, called AGROPOLIS. Administered by ARS's Office of International Research Programs, the 1,800-square-meter lab boasts a top-notch quarantine lab with three self-contained greenhouses. EBCL's staff at Montpellier and substations in Rome and Thessaloniki includes 30 scientists, lab technicians, and support personnel from America, France, Britain, Africa, and elsewhere.

EBCL is like an overseas outpost where the natural enemies of pests that scientists

From Montpellier's seaside airport, EBCL scientists routinely hop flights to the pests' points of origin in North Africa, the Middle East, Balkans, and Asia where natural enemies can be collected.

have collected can be reared, tested, packaged, and shipped stateside for use in classical biocontrol programs.

Says Quimby, "Our job is first to explore and find natural enemies of designated targets, and then characterize their identity, biology, and host range to make sure they're specific enough for introduction into the United States without causing problems."

To date, this has resulted in nearly 200 different biocontrol agents for use against at least 36 insect and weed species plaguing U.S. agriculture and natural habitats. These agents usually fall into one of four categories:

Parasites—including *Peristenus* wasp species, whose larvae develop inside *Lygus* bug nymph stages.

Predators—such as *Thanasimus formicarius,* a beetle that eats pine shoot beetles, a pest established in 12 states and Canada.

Pathogens—such as *Trichothecium roseum,* a fungus that infects the exotic shrub saltcedar, which in western states increases soil salinity and deprives native plants of water.

Weed Feeders—such as the weevils *Larinus minutus* on knapweeds and *Eustenopus villosus* on yellow starthistle.

One EBCL biocontrol success story is its research on seven parasites and one predator species from western Europe for control of alfalfa weevils. In the 1980s, these biocontrols were released in the United States and resulted in $90 million in yearly savings. In Texas's Rio Grande Valley and southern California, silverleaf whitefly numbers have declined since 1995, thanks to integrated pest management (IPM) tactics that include using insect growth regulators and parasite releases. Kirk and Guy Mercadier contributed further to the $1 billion pest's decline by finding 36 parasite species in explorations to 31 countries, including Spain, the United Arab Emirates, and Pakistan, from 1991 to 1997.

Currently, EBCL's top weed priorities include yellow starthistle, Russian

Entomologist Franck Hérard inspects gypsy moth larvae raised on an artificial diet. Larvae will be placed on oak trees to trap specific fly parasites, which will be sent to U.S. cooperators.

knapweed, saltcedar, Russian thistle, leafy spurge, hoary cress, perennial pepperweed, spotted knapweed, medusahead ryegrass, and rush skeleton weed.

The insect "hit-list" includes diamondback moth, pink hibiscus mealybug, wheat stem sawfly, gypsy moth, codling moth, apple leafrollers, olive fruit fly, grasshopper, locust, termites, Asian longhorned beetle, and *Lygus* bug.

Codling Moth and Leafroller

Kim Chen, a senior support scientist, is taking aim at codling moths and several leafroller species. These Eurasian insects are apple and pear pests in the northwestern United States. Chen's 1999 and 2000 surveys of three orchards in southern, central, and northern France revealed 65 parasite species, including braconid wasps and tachinid flies, that attack the pests.

One pest fighter is *Colpoclypeus florus,* a eulophid wasp that Chen and technician Jaime Lopez are rearing from collections made in French apple orchards. They will ship *C. florus* this spring to entomologist Tom Unruh, an ARS cooperator in Wapato, Washington, who wants to release a warm-climate strain of the wasp species. He will also receive a braconid wasp that parasitizes leafrollers. Unruh notes that previously released parasites of codling moth in Washington State have already become established.

Lygus Bug, Wheat Stem Sawflies, and Olive Fruit Flies

In addition to the *Lygus* bug, which damages alfalfa, cotton, strawberry, and many other crops, the scientists at EBCL are rearing parasites collected from wheat stem sawflies and olive fruit flies. Kim Hoelmer, entomologist, and Dominique Coutinot, senior support staff member, lead this project.

In Montana and North and South Dakota, sawflies cause roughly $100 million annually in yield losses. Female flies lay their eggs inside the wheat plant's stem, and emerging larvae eat their way down to the base of the stalk and excavate it. Weakened plants may topple over so they can't be harvested.

According to entomologist Tom Shanower, an ARS cooperator in Sidney, Montana, biocontrol is especially appealing against the sawfly because insecticides are too costly to use in wheat, and resistant wheats, called solid stem varieties, currently offer poor yields.

Hymenopterous wasps from Eurasia top the list of biocontrol candidates. Hoelmer's most recent search for them was in Uzbekistan. "This collection is now overwintering," he reports. "The sawflies and any parasitoids they harbor will emerge this spring."

Last fall, he and Coutinot went to Tunisia to find olive fruitfly parasites, of interest to Charles Pickett, a

California Department of Food and Agriculture (CDFA) cooperator. As the fly's population soars, Pickett sees an emerging threat to California's $33.9 million olive industry that will require biocontrol and other IPM tactics to minimize pesticide costs and environmental harm.

Before the fly's arrival, "California olive growers never had to worry about something getting into the fruit," says Pickett, who hopes to import new parasites in addition to one he's already test-released—*Psyttalia concolor.* Hoelmer says, "We found the flies at two different sites and made nice collections, from which we've already gotten two different parasitoids."

SCOTT BAUER (K9324-1)

A state-of-the-art quarantine facility is located next to the European Biological Control Laboratory. Dominique Coutinot, quarantine officer and support scientist, manages the facility under French Ministry of Agriculture rules.

Asian Longhorned Beetles

Franck Hérard, an entomologist, will be exploring Europe to identify natural enemies of cerambycid beetles. He hopes these enemies will be useful biocontrols for battling Asian longhorned beetles. Hérard is working in cooperation with Michael Smith, an entomologist with the ARS Beneficial Insects Introduction Research Laboratory (BIIRL), in Newark, Delaware.

The Asian longhorned beetle made its U.S. debut in New York's Central Park in 1996 and turned up 2 years later in Chicago's Lincoln Park. Between the two cities, 6,400 trees have since been removed, notes Smith. Unchecked, the pest poses a $3.8 billion threat to North America's hardwood and ornamental trees, according to USDA's Animal and Plant Health Inspection Service (APHIS).

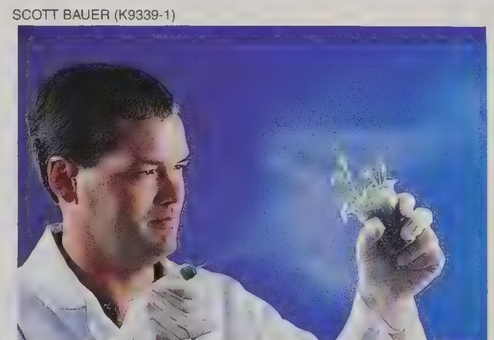
While Hérard scours Europe for promising new associations involving cerambycids and parasitoids, Smith's search for natural enemies will focus on the beetle's closest U.S. relatives. Chinese cooperators, meanwhile, will search for enemies that co-evolved with the pest in its native Asia.

Leafy Spurge, Russian Thistle, and Spotted Knapweed

The efforts of Rouhollah Sobhian, now retired from EBCL's biocontrol research team, included biocontrol agents for leafy spurge and Russian thistle (also known as tumbleweed). One agent, a gall midge called *Spurgia capitigena*, will be released by ARS cooperators in Montana against leafy spurge. Another promising biocontrol is *Gymnancylie canella,* a moth that in the caterpillar stage eats tumbleweed shoots and seeds.

Although an icon of the American West, tumbleweed is an invasive species some landowners consider a rangeland pest because of its prodigious seed production, flammability, wind-driven tumbling, and thorny stems. Says Sobhian, whose tumbleweed work is funded by CDFA, "It blows against fences, blocks waterways, and is said to cause accidents on highways."

SCOTT BAUER (K9339-1)



Plant pathologist Tim Widmer sprays a fungus isolated from yellow starthistle on a seedling of the same plant species. The fungus is harmless to humans.

Sobhian says more studies are needed on *G. canella's* overwintering habitat and host specificity before its value as a biocontrol can be fully ascertained.

Sobhian's successor, René Sforza, is renewing a spotted knapweed project in which he'll conduct explorations for biocontrols in Ukraine and Russia—areas formerly restricted during the Cold War. Sforza is also starting a new medusahead ryegrass project.

Saltcedar, Yellow Starthistle, and Other Invasive Weeds

Besides studying giant reed, plant pathologist Tim Widmer and technician Fatiha Guermache are looking for pathogens that cause root and foliar diseases on saltcedar, yellow starthistle, and other invasive weeds. "A plant pathogen," Widmer explains, "is most

commonly a fungus, bacterium, virus, nematode, or an abiotic factor, such as ozone or pollution, that continuously disrupts the plant's normal processes."

At CDFA, Widmer's cooperators are interested in pathogens as an alternative to controlling yellow starthistle with herbicides. First detected in the mid-1800s, the thistle now infests 10 million acres in California. Since spreading to other western states, it has become a pest of rangelands, prairies, vineyards, roadsides, and natural habitats. Uncontested, it displaces native grasses and plants that sustain livestock and wildlife. Widmer is currently testing more than 20 fungal strains and 45 bacteria isolated from thistle specimens and soils he collected in France earlier this year.

Pink Hybiscus Mealybug

Entomologist John Goolsby (of the ARS Australian Biological Control Laboratory) and Kirk are exploring Australia, Egypt, Hong Kong, Macau, and other places to collect natural enemies of pink hibiscus mealybug, a soft-bodied pest that attacks 200 different plants.

Because of its broad appetite and insecticide-blocking waxy coat, the bug's 1999 U.S. arrival in California caused much alarm. APHIS cooperators led by Dale Meyerdirk countered with timely parasite releases, including *Anagyrus kamali* wasps from the scientists' Australian collections. This action helped curb mealybug numbers by 98 percent, staving off millions of dollars in losses.

Genes Identify Good Inside the Bad

Molecular biologist Marie-Claude Bon and senior support scientist Nathalie Ramualde are working on genetic characterizations of biocontrol agents and their hosts. One objective is to customize biocontrol strategies based on genetic information about target pests and their natural enemies. They've also begun developing molecular diagnostic tools to detect and monitor parasitism and differentiate among parasites, including *Peristenus* wasps—some species of which have already been introduced into the United States and Canada for *Lygus* bug control.

SCOTT BAUER (K9342-2)

Technician Nicolas Crespy collects diamondback moth larvae from the undersides of wild cabbage leaves.

"We're developing a tool to detect the presence of parasitoid DNA extracted from the host nymphs," says Bon. They use genetic fingerprinting technologies similar to those used in crime labs, such as the polymerase chain reaction.

Formulating Hardy Fungi

Insect pathologist Guy Mercadier and Quimby, along with technician Nicolas Crespy, are testing new ways to formulate fungi into biopesticide products. They have a handy source of microbes to choose from. Since about 1988, Mercadier has amassed 1,000-plus strains of *Beauveria, Metarhizium, Paecilomyces,* and other fungi isolated from silverleaf whiteflies, diamondback moths, codling moths, African locusts, coffee berry borers, and other insect pests.

The researchers also developed a procedure to make *M. anisopliae* more resistant to drying. In arid regions like Africa's Sub-Sahara, for example, the sun can prevent this fungus from germinating inside its insect hosts. Called the "stabileze" method, their approach calls for shocking the fungus's spores with a dose of sucrose and ethanol, which removes up to 80 percent of cellular water.

"If we devise a good method of formulating a fungal pathogen that's resistant to UV light, high temperature, and degradation in storage," says Quimby, "this technology can probably be used with other fungi against other target insects in the United States."

Passing the Test

Once a parasite, predator, or pathogen has been deemed a worthwhile biocontrol candidate, EBCL scientists carefully package it for quarantined shipment to one of three ARS locations stateside.

The first stop for insects and insect pathogens is either the BIIRL or ARS' Stoneville (Mississippi) Research Quarantine Facility. Weed pathogens go to ARS' Foreign Diseases-Exotic Weed Science Unit, in Fort Detrick, Maryland.

New arrivals must pass a stringent inspection before they're sent to EBCL's cooperators, which include other ARS labs, state agriculture departments, other USDA agencies (including the Forest Service) and non-USDA agencies in the Department of the Interior. The list includes state and land-grant universities, with cooperators in Oregon, California, North Dakota, Montana, Wyoming, Colorado, Illinois, and Kentucky.—By **Jan Suszkiw,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at http://www.nps.ars.usda.gov.

To reach scientists mentioned in this story, contact Jan Suszkiw, USDA-ARS Information Staff, 5601 Sunnyside Avenue, Beltsville, Maryland 20705-5129, phone (301) 504-1630, fax (301) 504-1641, e-mail jsuszkiw@ars.usda.gov. ◆

Bt Corn Not a Threat to Monarchs

Last summer, when entomologist Mark K. Sears was researching whether *Bt* corn posed a risk to monarch butterflies, he noticed that two monarchs had hit his car's windshield as he drove to test fields around Ontario, Canada.

"That's probably more monarchs than were lost that day because of *Bt* corn, according to findings in our studies," says Sears, with the Department of Environmental Biology at the University of Guelph.

Sears is part of a group of scientists coordinated and partially funded by the Agricultural Research Service who have spent 2 years investigating whether *Bt* corn is a threat to monarch butterflies.

Bt corn contains genes from the bacterium *Bacillus thuringiensis* so that the plant will produce proteins to protect itself against insect pests such as the European corn borer. This reduces the amount of insecticide farmers need to apply. Since *Bt* corn was introduced to the marketplace, use of the insecticides recommended for European corn borer control has decreased from 6 million acre treatments to slightly over 4 million in 1999, a drop of about one-third, according to the Environmental Protection Agency.

Although *Bt* corn was approved in 1995, new concern about possible risk was raised when a note published in a May 1999 issue of *Nature* suggested that *Bt* corn could harm monarch butterflies when the caterpillars were given no choice but to feed on milkweed leaves heavily coated with *Bt* corn pollen.

Monarch caterpillars feed exclusively on leaves of milkweed plants, which grow in and around cornfields. But during the 1 to 2 weeks a year that corn pollen is shed, it can be blown onto the milkweed leaves.

In response, ARS organized a series of workshops that encouraged butterfly biologists, corn researchers, ecologists, entomologists, and other experts to work together to determine whether a risk actually existed. During a February 2000 workshop, a group of scientists from government, universities, industry, and environmental groups prioritized specific research needs. The idea was to ensure that all the most important questions were covered. In addition to funds already assigned, ARS contributed $100,000 to a grant pool, which was then matched by industry, to fund the research.

The Real Risk to Monarchs

Monarch butterflies are popular insects. Children and adults all over the country help track this butterfly's annual migration across North America. They are not currently an endangered or threatened species. Habitat destruction; mowing of highway right-of-ways, ditches, and pastures, which destroys milkweed; collisions with vehicles; and insecticides all play a part in reducing monarch populations. But the most common fate for monarch caterpillars is being eaten by another insect. Fewer than 10 percent of monarch caterpillars make it to adulthood each year.

The collaborations established at the workshops continued throughout the research process. For example, the group agreed early on to use similar experimental designs and methods, such as how to handle the pollen. This ensured that data collected by different scientists would be compatible.

"Being able to pool data gave us much larger, more reliable sample sizes, so we could develop the best scientific answers to the question of risk," says ARS entomologist Richard L. Hellmich, with the Corn Insects and Crop Genetics Research Unit, Ames, Iowa. Hellmich was the lead ARS scientist on the project.

Cooperation even extended to how the research was published. All the researchers funded from the special grant pool got together and divided the data into logical sections and agreed to submit all manuscripts together to a single scientific journal. Publishing the exposure, toxicity, and risk-analysis studies at one time in one journal provided the most complete picture possible of whether any risk actually existed.

Two Big Questions

To determine whether the concern about *Bt* corn was valid, two major questions needed to be scientifically answered: "Exactly how much *Bt* pollen does it take to cause toxic effects in monarch caterpillars, and what are the chances caterpillars will encounter that dose under natural conditions?" Hellmich says.

PEGGY GREB (K9649-1)

Entomologist Rich Hellmich (right) and technician Randy Ritland collect milkweed leaves near pollinating corn.

PEGGY GREB (K9645-1)

Student aid Stacy Van Loon releases a monarch butterfly into a breeding cage. The butterflies consume artificial nectar from the flower-shaped feeder.

PEGGY GREB (K9644-1)

A newly emerged monarch butterfly feeds on an artificial feeder.

Toxic Anthers

When Hellmich and his colleagues began their *Bt* corn/monarch butterfly laboratory studies, they encountered a much higher level of *Bt* protein in the pollen than expected. They checked several possible explanations, including inspecting their methods for pollen collecting and preparation.

"We found that some of the pollen we were using in our tests was contaminated by ground-up or fractured anthers," Hellmich says. Anthers—the organs at the end of plant stamens that produce pollen—have a much higher level of *Bt* protein than does pollen itself. "But simply passing the pollen through a fine screen before using it removed the anthers," he adds.

The problem of anther contamination may explain the toxic results in some earlier studies, Hellmich points out. But that left the question of whether caterpillars might eat the high-*Bt*-containing anthers under natural conditions.

"Anthers have commonly been found on milkweed leaves within cornfields, but none of them were fractured. Fractured anthers appear to be an artifact of pollen processing in the laboratory," Hellmich says.

Then they looked at caterpillar-anther interaction. "Our preliminary results show that small larvae avoid anthers. With the size difference, it would be like a person trying to eat a city bus," Hellmich points out. "Wind and rain also readily dislodge anthers from milkweed leaves, making it less likely that caterpillars will encounter anthers."

First, the scientists assessed the feeding behavior of monarch larvae—caterpillars—to see whether *Bt's* presence on milkweed leaves influenced their weight and survival. Pollen from six *Bt* corn types—BT11, MON810, CBH351, DBT418, TC1507, and BT176—was tested along with no-pollen and non-*Bt*-corn-pollen controls.

"We looked at larval weight and larval survival and found it took large amounts of pollen to get any statistically significant effect," Hellmich says.

Eating leaves with pollen coating densities below 1,000 grains/cm^2 had no effect on caterpillars' weight or survival rate. Above 1,000 grains/cm^2, caterpillars were smaller than those from the control treatments, but their survival rate was no different from that of controls.

One type of *Bt* corn—BT176—did show some harm to larvae at pollen levels of 10 grains/cm^2. BT176 was the earliest *Bt* corn developed and was quickly supplanted by other types. It has never been planted on more than 2 percent of all corn acres and is likely to be completely phased out by 2003.

Once the scientists knew how much *Bt* corn pollen it took before monarch caterpillars showed any ill effect, the second question was how often are they exposed to pollen levels above 1,000 grains/cm^2 under natural conditions?

To find out, the researchers established corn pollen density and distribution patterns on milkweed leaves near cornfields. Hellmich's team set up lines of collecting devices at seven different fields, from the edge of the field to 600 feet away, in all four compass directions.

The researchers measured pollen deposition three ways. They put out tubes holding cuttings of milkweed stems with two leaves, whole potted milkweed plants, and microscope slides coated with glycerin. Sampling lasted about 10 days—covering peak pollen production periods.

"We found that, on average, less than 30 percent of the pollen that corn produces ends up on milkweed leaves, even when conditions are perfect, and most of that gets deposited on milkweed within the cornfield," Hellmich says of his field studies in Iowa.

PEGGY GREB (K9642-1)

A large monarch caterpillar feeds on a common milkweed plant.

PEGGY GREB (K9647-1)

Entomologist Les Lewis (left) and technician Keith Bidne observe a group of newly emerged monarch butterflies.

In Ontario, Sears conducted similar field studies of pollen deposition and found the same pattern. Other pollen studies by University of Maryland and University of Nebraska researchers also confirmed the pattern and extent of pollen distribution.

Data pooled from Iowa, Nebraska, Maryland, and Ontario showed that the average *Bt* corn pollen density on milkweed leaves inside cornfields was about 170 grains/cm^2, and it rarely went above 600 grains/cm^2.

"These pollen densities mean monarch caterpillars inside cornfields will encounter pollen levels exceeding 1,000 grains/cm^2—the lowest observable effect dose—less than 1 percent of the time," Hellmich points out.

Many factors contribute to keeping pollen density low. Corn pollen is relatively heavy, so it doesn't blow far; higher milkweed leaves tend to shelter lower leaves; and rain washes pollen off of milkweed leaves easily, Hellmich says.

Given the low toxicity of *Bt* corn pollen and the low rates of exposure, the effect of *Bt* corn pollen from common commercial hybrids on monarch butterfly populations is negligible. "Furthermore, you need to compare the potential for risk to monarchs from *Bt* corn with the alternative, which is chemical insecticide use," Hellmich says.—By **J. Kim Kaplan,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at http://www.nps.ars.usda.gov.

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Ousting Pink Bollworm From Cotton Fields

Pink bollworms emerging from a damaged cotton boll.

"Pink bollworm is the most important cotton pest in the world," entomologist Thomas J. Henneberry declares. "It's found in almost every cotton-producing country and has caused millions of dollars of damage and lost acreage in the last 35 years in the United States."

Henneberry is the research leader for ARS' Western Cotton Research Laboratory in Phoenix, Arizona. Some of the findings from years of pink bollworm research at this lab are being used by the National Cotton Council (NCC) Pink Bollworm Action Committee in a cooperative program to eradicate the pest.

In its adult, or moth, stage, the pink bollworm lays its eggs on cotton bolls. The eggs hatch into larvae that eat the cottonseeds and damage and discolor the fiber. According to the NCC—a trade organization representing the U.S. cotton industry—total costs to cotton producers are more than $21 million annually in prevention, controls, and lost yields.

There have been many attempts to get rid of this pest, but those involved with the program think they may finally succeed. ARS conducted the initial pink bollworm research in Hawaii back in 1915, since most believed the insect would enter the U.S. mainland eventually. Two years later, it entered Texas in infested cottonseed. By 1965, it had spread throughout southern California and the southwestern United States.

ARS has studied four general eradication approaches over the years. A combination of these technologies will be used in the program, which will rely on close partnerships with cotton producers. The first is to create a host-free period by shortening the growing season. This would make it harder for the pest to survive to the following season.

A second facet of this program is transgenic pest-resistant cotton. ARS researchers are working with industry to develop cotton that would not be destroyed by pink bollworms and other lepidopteran insect pests.

The third technique is to disrupt mating. Female pink bollworms release a scent so that the males can find them to mate. ARS and other researchers have developed methods of using a powerful version of this scent that, when released in cotton fields, confuses the males and makes finding the females nearly impossible.

The final part of the program will be release of sterile pink bollworm moths into cotton fields. But this only works when the population of pink bollworms is already low, says Nate Dechoretz, chief of integrated pest control for the California Department of Food and Agriculture (CDFA). Key ARS rearing research, with modifications by USDA's Animal and Plant Health Inspection Service, has allowed for large-scale moth production. Dechoretz's CDFA branch is capable of growing up to 30 million moths a day for the sterile-release program.

Essential to the program's success will be pink bollworm population monitoring, transgenic cotton resistance management, and data analysis and interpretation, Henneberry says.

The eradication program is already under way and is proposed for three phases in different locations in the southwestern United States and northern Mexico. It will be completed in 2004 or 2005.—By **David Elstein,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at http://www.nps.ars.usda.gov.

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Pink Bollworm Bibliography

The longstanding nature of the pink bollworm problem worldwide and the likely development of areawide management programs in the future prompted three ARS scientists to produce a comprehensive unannotated bibliography of world literature on the pink bollworm. Single copies are available, while supplies last, from Steven E. Naranjo, USDA-ARS-PWA, 4135 East Broadway Rd., Phoenix, AZ 85040-8803. The bibliography is available as an Adobe Acrobat pdf at http://www.ars.usda.gov/is/np/pinkbollworm/pinkbollwormintro.htm and is searchable online at http://www.wcrl.ars.usda.gov/biblios/pbw/pbwbiblios.html where the authors will also post yearly addenda.

We Don't Cotton to Boll Weevil 'Round Here Anymore

The farmer said to the merchant
I need some meat and meal.
Get away from here, you son-of-a-gun,
You got boll weevils in your field.
Going to get your home, going to get your home.
—Carl Sandberg's version of "The Boll Weevil Song," 1920

Agricultural Research Service boll weevil scientists have almost succeeded in working themselves out of a mission in the best possible way—by having helped to create a program that is closing in on its goal of eradicating the boll weevil from the United States.

Boll weevil eradication has been a monumental success story, often compared in scope to the elimination of the screwworm in the United States.

USDA History Collection. Special Collections, National Agricultural Library (K10298-1)

Dedicated in 1919, the Boll Weevil Monument, in Enterprise, Alabama, is symbolic of just how important the boll weevil is in the South.

B.R. Coad, U.S. Dept. Of Agriculture (K10299-1)

Mule-driven cart being used in 1919 in Scott, Mississippi, to dust for boll weevils.

For more than 100 years, boll weevils wreaked havoc on the U.S. cotton industry. Since its entry into the United States from Mexico in 1892, the insect scientifically known as *Anthonomus grandis* Boheman spread throughout the South, forcing radical economic and social changes in areas that had been almost completely dependent on cotton production. Many experts consider the boll weevil second only to the Civil War as an agent of change in the South. Over the years, estimates of yield losses and control costs due to the boll weevil total more than $22 billion.

Hope for stopping the boll weevil in its beetle tracks was relatively bleak until the 1970s, when research by ARS and universities like Texas A&M, Louisiana State University, Mississippi State University, and others evolved into a complex of tools that had the potential to remove this foreign invader from the country.

Today, the boll weevil is in full retreat throughout the South. Since the program was first pilot tested in 1978 along the Virginia-North Carolina border, the pest has been eradicated from more than 6 million acres. There are active eradication programs in various stages of completion on over 9 million acres in 17 U.S. states and in parts of northern Mexico. Where the boll weevil has already been eliminated, cotton has made a significant return, especially in traditional growing areas like the Carolinas, which were declared essentially boll weevil free in 1986.

"There haven't been many pest programs that have been as successful as this one. And without this program, there would be very little, if any, cotton grown in the Southeast today," explains Jim Brumley, executive director of the Southeastern Boll Weevil Eradication Foundation. "The real key to our success is that we have had good research to apply. We couldn't have developed or carried out this program without the research that was done by ARS and others."

Cotton grower foundations like the one Brumley heads are the organizational backbone of the boll weevil eradication program, which is funded about 70 percent by cotton growers and 30 percent through USDA's Animal and Plant Health Inspection Service (APHIS). As the program moves toward completion, ARS continues to provide critical research to support field operations.

Bill Grefenstette, APHIS' national coordinator for boll weevil eradication, sees the cooperation among all those involved in the program as key to making boll weevil eradication a reachable goal. "I'm not sure many people realize how big an undertaking this program really is—taking on one of the most important insect pests in our history and involving 17 states and more than 15 million acres," Grefenstette says. "The overall program, including ARS' linchpin research—along with the Cooperative State Research, Education, and Extension Service, the universities, Extension Service, the states, the industry, the growers, and thousands of dedicated

ROB FLYNN (K2742-10)

ROB FLYNN (K2688-13)

Left: Boll weevil on a cotton boll.
Above: ARS entomologist Bill Dickerson (left) and cotton farmer Marshall Grant examine a boll weevil pheromone trap on Grant's farm in North Carolina. These traps have been key to the success of the eradication program. (Photo taken in 1987.)

employees—has been a model of how folks can cooperate in setting a critically important goal and then working together to accomplish it."

Seeds of Success

The seeds of today's successful eradication program were planted in the late 1950s, when two factors coincided. The U.S. cotton industry, facing serious economic challenges, focused on elimination of the boll weevil as one of the best ways to reduce its enormous costs.

At the same time, a major revolution was under way in entomology and insect pest control, started by ARS entomologist Edward F. Knipling. He had devised an areawide pest-management program that was already showing tremendous success eradicating screwworm from the United States.

Knipling's success was convincing evidence that it was feasible to eliminate a pest by striking at it through its biology. Even the format for the boll weevil program took its direction from the screwworm program. The Southwest Animal Health Foundation, which was formed to facilitate screwworm eradication in the Southwest, later served as a template for the boll weevil eradication foundations.

Joining the commitment to do away with the boll weevil, ARS built the Boll Weevil Research Laboratory (BWRL) in Starkville, Mississippi, in 1961. In addition, boll weevil research was significantly strengthened at other ARS labs.

These researchers came up with two linchpins of today's successful eradication program: an effective, inexpensive detection trap and a pheromone lure.

To identify the pheromone, first a way to test candidate compounds on live boll weevils—a bioassay—needed to be developed. That work was done between 1965 and 1969 by Dick Hardee, now research leader of ARS' Southern Insect Management Research Unit in Stoneville, Mississippi.

"Three years after I came up with a good bioassay, Jim Tumlinson called me up and asked if I had a good crop of boll weevils ready for an assay because he was pretty sure he had come up with the pure pheromone," Hardee says. Now research leader of the ARS Chemistry Research Unit in Gainesville, Florida, Tumlinson was then a graduate student working for ARS at the Mississippi State University facility.

What Tumlinson found were four compounds that, in combination, were as attractive to female boll weevils as live males were. None of the compounds was active alone, which had made isolation and purification more difficult.

Isolating a pheromone in 1969 was still new research territory, and the equipment was not very sensitive. Tumlinson had to isolate about 10 milligrams of each compound to characterize it; today, compounds are routinely identified from 1 microgram and sometimes even from 1 nanogram, a 10,000- to 10-millionfold reduction in sample requirements.

Tumlinson recounts, "To get enough of each compound to determine the structure, I eventually extracted and steam-distilled 54 kg of boll weevil feces."

Hardee christened the pheromone complex Grandlure, which continues to be one of the four or five most effective pheromone lures in commercial use.

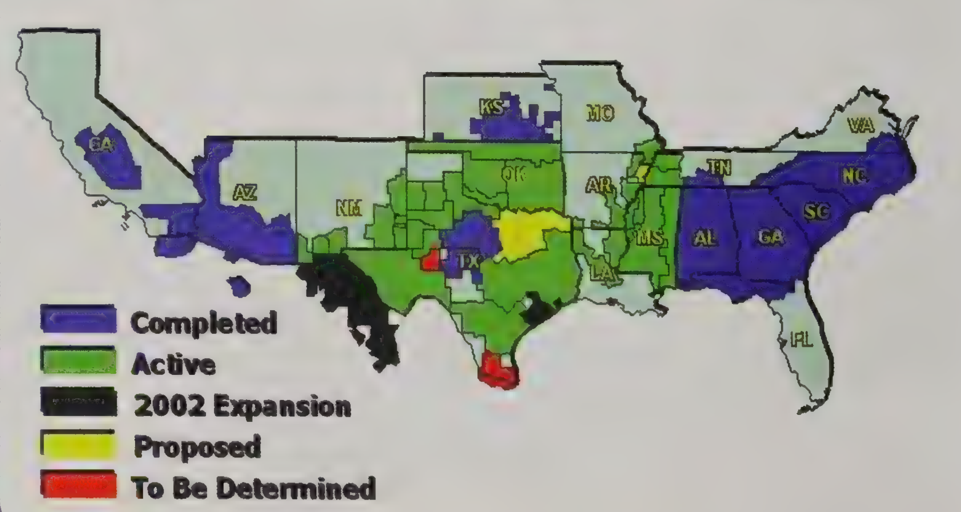
During the same period, Joseph E. Leggette, who was then with the BWRL, made a quantum leap forward in trap design, creating an effective live trap from an inverted floral paperliner. Several more generations of refinements were made by a number of ARS researchers until they came up with the design still being used today. More than 20 million of these traps have been used in the United States since1987.

"Growers had just been waiting for us to come up with these two tools to dig into planning an eradication program," Hardee explains. With a trap and a lure, insecticide use could be targeted to boll weevil appearance in cotton fields.

Using Biology Against the Boll Weevil

The boll weevil eradication program depends primarily on detection and carefully targeted insecticide use, unlike the screwworm program to which it is often compared but which depended on biological control—releasing sterile male insects to prevent reproduction. But it was

Boll Weevil Eradication in the United States and Mexico



Knipling's advocacy in both programs for research to understand the two pests' biology and ecology that laid the groundwork for so much of the boll weevil eradication program's success.

Researchers had to learn and are still learning about such traits as how far boll weevils will travel, especially in the face of strong winds; what other plants attract them; and the insects' diapause, or winter dormancy.

An ARS team led by Edwin P. Lloyd at BWRL focused on finding a way to use diapause as an effective time to reduce the boll weevil population while reproduction slows. In 1968, the team conducted a 5,000-acre "reproduction-diapause" control technique demonstration in Mississippi. It successfully used for the first time a system of targeting insecticide applications to diapausing boll weevils and monitoring pheromone traps.

ARS researchers then gathered all the available research—theirs and that of many other scientists—and built a model of an areawide eradication system that would eliminate boll weevils. The next step was to find a large cotton-growing area, convince the growers to participate, and start eradicating boll weevils.

A pilot test of control techniques was run in southern Mississippi and adjacent cotton growing areas in Alabama and Louisiana from 1971 to 1973. For the first time, it was shown that boll weevil eradication was finally technologically feasible.

Then, with the support of the National Cotton Council and the approval of the majority of local growers, a 3-year Boll Weevil Eradication Trial began in Virginia and northern North Carolina in 1978, conducted by APHIS with research support from ARS.

The trial proved to be an overwhelming success and established the operational strategy for all future programs as they spread throughout the South.

Cotton farmer Marshall Grant and his wife in 1987.

FEAR NO WEEVIL

Marshall Grant and his family have been growing cotton in North Carolina since the 1780s. But boll weevils almost put him out of business between the environmental and economic costs of control.

"We were spraying insecticides every 5 days until the bolls were mature. Now that boll weevils have been eradicated from the Carolinas we're down to an average of one, maybe one and a half sprayings per year for other pests," Grant says. "The eradication program has made the difference between there being cotton production here and there not being any cotton production in North Carolina."

"The model that ARS developed has been the key to success in each new state as growers voted to go for eradication," says Charles T. Allen, program director of the Texas Boll Weevil Eradication Foundation. "There has always been so much politics and contention in cotton production. If the plan hadn't come from ARS and other collaborators, I'm not sure that growers would have had as much confidence in the possibility of eradication."

Allen worked for the Texas Agricultural Extension Service with cotton growers for 20 years before joining the Texas foundation. Until he saw the eradication program in use, he could hardly believe that he would ever see the day when the boll weevil would be eliminated. "Now I think I will see it to completion before the end of my career," Allen says.

Costs and Payoffs

At one time, cotton growers applied more than 41 percent of all insecticides in agricultural use; they regularly sprayed their cotton as many as 15 times a season. In the first season of an eradication program, an average of seven or eight insecticide applications are timed for the fall, just before diapause. In subsequent years, insecticide application is based on finding boll weevils in traps, with an average of five applications in the second year and only two in the third year.

"Eradication is expensive to begin with, but the payoff is tremendous as the program moves along," explains Frank Carter of the National Cotton Council of America. "Early on, our studies showed about a 12:1 benefit-to-cost return to the cotton industry for every dollar invested in eradication."

But the economic payoff is really just starting, he points out. Pesticide costs continue to decrease as eradication succeeds in more and more states. There will be generations of cotton growers who

may never have to spray for boll weevils. "Every year that happens will be a payoff of this program," Carter adds.

One ARS contribution in the mid-1990s that helped the economic viability of the program was finding that lower doses of malathion, the only insecticide currently used for eradication, are just as effective as higher ones. This decreased the cost of the program dramatically.

In addition to significantly lower control costs, there are environmental benefits of trading weekly spraying by individual growers for nationwide eradication. By reducing the amount of pesticides being used as the eradication program succeeds, more beneficial insects survive to protect cotton from other pests. As the ecosystem changes, researchers have had to conduct additional studies to ensure that no new problems are created for growers in place of the boll weevil.

There have even been some unexpected payoffs of ARS boll weevil research. In January 2002, after checking out new ARS research on the boll weevil's inability to survive in or on compacted cotton bales, APHIS and Peruvian agricultural officials reached an agreement to remove the mandatory fumigation of U.S. cotton bales with methyl bromide to prevent spread of boll weevil. Curtailing fumigation will significantly reduce the cost of exporting cotton to Peru. A similar agreement has been reached with Colombia, and discussions are being held with Pakistan and other countries.

Finishing the Job

Although the eradication foundations are closing in on final success, there remain several major issues on which ARS continues to conduct research, says entomologist Dale W. Spurgeon with the ARS Areawide Pest Management Research Unit, College Station, Texas. Spurgeon is also ARS' lead scientist for the agency's boll weevil efforts.

One issue is the impact of conservation tillage, also called no-till, on the eradication program. "We have some indications that boll weevil survival is different in no-till," Spurgeon says. "We need to understand and adapt to the effects of no-till as it becomes a more common practice in eradication zones."

Another issue left for research is completing a DNA fingerprint library of boll weevils so that when one reappears in an eradicated area, its source can be identified. This will also help identify the most effective locations for detection traps.

And the effect of weather, especially of extreme events like hurricanes, on the potential for the boll weevil to reenter the United States from Mexico also needs study.

What a Difference No Boll Weevil Makes

The year before boll weevils marched into Georgia in 1915, the state produced 2.8 million bales of cotton. Less than 10 years later, Georgia's annual cotton production had fallen to 600,000 bales. By 1983, Georgia cotton production was down to 112,000 bales harvested from 115,000 acres.

But in 1987, Georgia began a boll weevil eradication program. A decade later, in 2000, cotton production in Georgia had rebounded to 1.66 million bales. In this one state alone, the cotton industry, including farms, gins, warehouses, cottonseed oil mills, and textile mills, provides 53,000 jobs and has an overall economic impact of more than $3 billion each year.

ROB FLYNN (K2689-16)

The presence of beneficial insects such as the lady bird beetles on this cotton bloom are testimony to the benefit of reduced pesticide use as a result of the boll weevil eradication program.

"Understanding how and why control measures work is essential to the continued progress of eradication," Spurgeon says. "If we don't know why something happens, how can we be ready to correctly tweak the eradication model to deal with it?"

But Spurgeon and the other ARS boll weevil researchers have created a Catch-22 for themselves. The program has just about eradicated them out of places to do their research. Once the boll weevil has been eliminated from a state, they can't do research there anymore. That's because they they don't want to inadvertently reintroduce the insect.

"I can't say I'm too upset at the prospect, since complete eradication has been our goal for so long," he says.—By **J. Kim Kaplan,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at http://www.nps.ars.usda.gov.

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Beating Summertime Insect Woes

Today, thanks to ARS research, consumers have some protection against mosquitoes.

Since World War II, ARS research has focused mainly on two types of mosquito protection for military personnel and the general public: treated fabrics and insect repellents for use on skin. In the mid-1950s, ARS researchers discovered the mosquito victim's dream: DEET, now a widely used repellent. Today, millions of people worldwide apply DEET to their skin; it's the main active ingredient in about 200 commercial products.

However, "DEET is very oily and has an unpleasant smell," says Donald R. Barnard, head of ARS' Mosquito and Fly Research Unit at Gainesville, Florida.

"Currently, there is no alternative as effective as DEET with the qualities that would be pleasing to consumers."

Finding such an alternative has been a primary goal of ARS scientists at Gainesville, where researchers have tested 16,000 new candidate repellents in the last 20 years—with several new ones in store for the future. One group of synthetic repellents under testing now, called piperdines, appears very promising as a possible DEET alternative.

ARS scientists, in cooperation with the U.S. Army, developed fabric treated with the insecticide permethrin for use in clothing that can be used as a barrier to prevent insects from biting U.S. troops who travel abroad. In many underdeveloped countries, tropical diseases transmitted by mosquitos are a problem—particularly malaria and dengue fever.

World Health Organization statistics report about 4 million malaria cases each year and about 1 million deaths, while about 24,000 deaths occur each year from dengue-related illnesses. About 2.5 billion people are currently at risk worldwide for dengue, and the potential for exposure to mosquito-transmitted diseases could increase if temperatures increase worldwide.

Today, the U.S. Department of Defense uses a two-pronged strategy: DEET and permethrin-treated clothing—called the personal protection system—against mosquitos and other health-threatening pests that could lead to possible disease exposure.

So what is in store for the next millennium? ARS researchers are looking at what makes us so tempting and tasty. They are trying to identify and understand what scents humans and animals emit that say to mosquitos, "Hey, free lunch!" They hope to take this knowledge and use it to develop new attractants, which could be used for monitoring or in combination with toxic baits to kill the pests.

"Ultimately, in the next millennium, we're trying to develop nonpesticidal control methods, which include biological, behavioral, biochemical, and genetic control technologies," says Barnard.—By **Tara Weaver-Missick**, ARS.

This research is part of Arthropod Pests of Animals and Humans, an ARS National Program (#104) described on the World Wide Web at http://www.nps.ars.usda.gov/programs/appvs.htm.

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SCOTT BAUER (K7243-7)

In the mid 1950s, ARS scientists discovered DEET. Though it is still the main active ingredient in about 200 commercial products, new insect repellents are being researched today.

Update

Hot on the Trail of Fire Ants

Red imported fire ants keep adapting to life in the United States. They keep spreading farther and farther north from their foothold in the Southeast, often overtaking native ants along the way to become the dominant species.

But scientists at ARS' Center for Medical and Veterinary Entomology (CMAVE) are hot on their tiny trails, developing new ways to keep their numbers manageable.

Researchers with the Imported Fire Ant and Household Insects Research Unit in Gainesville, Florida, have made many discoveries since the late 1970s in their efforts to control fire ant populations. Their strategy is to reduce the amount of chemical treatments needed by developing self-sustaining biological control agents.

The red imported fire ant, *Solenopsis invicta,* was accidentally introduced into the United States in 1929 from South America. Fire ant population there is only 20 percent of that here, probably because North America lacks natural enemies of the ant. They are particularly disruptive because of their large numbers and painful sting; about 40 percent of people in infested areas are stung each year. Fire ants dramatically reduce populations of native ants, other insects, and ground-nesting wildlife. They also damage many crops and much electrical equipment. Damage and control costs are estimated to be more than $6.5 billion a year.

Red imported fire ants currently infest more than 320 million acres in 12 southeastern states and Puerto Rico and have recently spread to California and New Mexico. Although fire ants keep moving into new areas, a recent ARS model predicts that they will not be able to survive in areas where soil temperatures drop to near freezing for more than 2 to 3 weeks. Black imported fire ants, *Solenopsis richteri,* introduced to the United States in 1918, are less widespread. A hybrid looks the same as the red ants, so laboratory tests are needed to tell them apart.

A single-queen colony can have 250,000 workers.

Effective Infections

Two pathogens from South America are known to infect fire ant colonies, and research is continuing into their effectiveness as biological control agents. CMAVE researchers were the first to discover *Thelohania solenopsae* in the United States in 1996. This single-celled protozoan parasite infects colonies when workers transfer it to the queen, possibly through food exchange. It reduces the queen's weight, and she lays fewer eggs. Colonies die out after 9 to 18 months.

CMAVE researchers learned they could infect colonies by introducing brood infected with *T. solenopsae*. Since fire ants will accept brood from foreign colonies, the parasite is now established in many regions. But because the transfer of live larvae and pupae is very tedious and not entirely effective, experiments are under way to see which life stages best transmit the pathogen to the queen. Entomologist David Oi recently discovered in fire ant pupae a new *T. solenopsae* spore type that he believes might be capable of initiating infection.

Some fire ant colonies have only a single queen, while others have many. A single-queen colony can have 250,000 workers, whereas a multiple-queen colony can have twice that number. Multiple-queen colonies are easier to infect with *T. solenopsae* because they will adopt and raise infected brood from other colonies. But, on the downside, says Oi, they are harder to control because their large populations limit the pathogen's impact.

"Single-queen colonies are more suspicious of outsiders, but they will raid other colonies and take their immature ants," says Oi. "So, we can establish an infection in single-queen colonies, but it dies down after awhile."

The researchers are studying another pathogen, *Vairimorpha invictae,* that is more lethal but also more rare than *T. solenopsae.* (See "Ouch! The Fire Ant Saga Continues," *Agricultural Research,* September 1999, p. 4.) *V. invictae* is hard to keep alive in the laboratory, so the scientists must occasionally have infected colonies sent from ARS' South American Biological Control Laboratory in Buenos Aires, Argentina—where it is being studied in the field—to examine the pathogen under quarantine in Gainesville.

A new disease has been discovered by CMAVE insect pathologist Roberto Pereira. It's called yellow head disease because of the yellow-orange color ant heads and other body parts turn when infected with the spindle-shaped spores. A protozoan from the genus *Mattesia*

was present in 34 percent of sites and 8 percent of nests studied in Florida. The pathogen causes extreme changes in appearance, but its full impact as a potential biological control agent is still under investigation.

David Williams, head of the Imported Fire Ant and Household Insects Research Unit, is also searching for viruses to use against the ant. "Back in the late 1970s, a researcher looking at red imported fire ants found a virus-like particle, but it died in the lab," Williams says. "There are viruses in ants, but finding them is tedious and laborious work. They can be extremely virulent if you get the right one." He believes this untapped area has great potential for new discoveries, and he hopes to bring a molecular biologist to their team soon to aid in the search.

Williams is also evaluating a parasitic ant, *S. daguerrei,* under quarantine. It is found only on fire ants in Argentina and Brazil. The parasite doesn't produce workers, so it relies on its host, taking resources from the queen while the colony feeds and maintains its brood. Mound densities were 33 percent less in sites with the parasitic ant, and the number of fire ant queens was 47 percent less in parasitized colonies.

Terror from Above

Fire ant decapitating phorid flies, from the genus *Pseudacteon,* are natural enemies of imported fire ants. Just their presence keeps frightened ants from leaving their mounds. That alone is good news because it forces a shift in when the fire ants forage and allows native ants to compete better.

A female fly hovers over a mound before she pinpoints a victim, dive-bombs it, and deposits one of her eggs inside it. The egg, as researchers at the center discovered, eventually produces a larva that eats its way to the head and causes it to fall off. Entomologist Sanford Porter estimates there are about 20 types of *Pseudacteon* flies that attack fire ants. They could be used to complement each other in control efforts.

So far, two phorid fly species—*P. tricuspis* and *P. curvatus*—have been

PEGGY GREB (K10233-1)

Entomologist Sanford Porter inspects fire ants hiding under cups inside a large attack box for rearing fire ant-decapitating flies.

PEGGY GREB (K10229-1)

The fire ant queen on the bottom right is healthier and much heavier than the *Thelohania solenopsae*-infected one on the top left.

Entomologist David Williams examines an image of a fire ant worker containing a cyst of the microbial pathogen *Thelohania solenopsae.*

PEGGY GREB (K10222-1)

released and established in the United States. *P. tricuspis* took hold and is expanding at the rate of 10 to 20 miles a year. *P. curvatus* works better on black imported fire ants and the hybrid than on red ones. It was imported from Argentina and is being used in Alabama, Mississippi, and Tennessee. A third species, *P. litoralis,* works especially well on the red ants and has been approved for release in field studies.

Porter and colleagues at ARS' Biological Control and Mass Rearing Research Unit, located near Starkville, Mississippi, developed an attack box that has greatly improved their ability to mass-rear phorid flies. The boxes are held under environmental conditions that strictly mimic the flies' natural habitat. Specially designed cups are alternately raised up and down within the box in a cycle every 10 minutes, causing the ants to run from one cup to the other. This action allows the phorid flies to easily attack and parasitize them. New flies are continually released into the main attack box from an attached holding box at one end to maximize the amount of eggs deposited.

PEGGY GREB (K10225-1)

Entomologist David Oi collects infected fire ants from a colony decimated by the fire ant pathogen *Thelohania solenopsae*.

Because CMAVE reached its capacity to rear the phorid flies, an initiative to mass-produce them was launched with ARS, USDA's Animal and Plant Health Inspection Service (APHIS), and Florida's Department of Agriculture and Consumer Services. The new facility in Gainesville will eventually produce 6,000 to 12,000 flies a day.

Social Insects Suffer Uninvited Guests

Chemist Robert Vander Meer studies semiochemicals, such as pheromones, in an effort to modify ant behavior and control the pests. Social insects like fire ants use semiochemicals to communicate with each other and influence activities. Vander Meer wants to see how the ants use these compounds and then disrupt the processes.

One reason fire ants are so troublesome is that they commonly short-circuit electrical equipment of all kinds. Of course, they electrocute themselves in the process, and large numbers of dead ants are sometimes found piled up in electrical mechanisms. Vander Meer and Porter discovered that electrocution causes the ants to release alarm pheromones that attract other ants as well as phorid flies. In response to these pheromones, phorid flies lay more eggs in ants, and fly production increases by 15 to 20 percent. This knowledge will help them develop new ways to use alarm pheromones in biological control programs.

Vander Meer is involved in other fire ant pheromone projects. He found that fire ants use their stinger not only for defensive purposes but also for pheromone dispersal. His knowledge of how fire ants use semiochemicals to recognize intruders enabled scientists to decipher how two parasites (a beetle and a wasp) are able to infiltrate aggressive colonies. Pioneering work revealed how fire ants locate food and recruit other workers to the source with pheromone trails.

PEGGY GREB (K10236-1)

Entomologist Roberto Pereira and technician Susan Reidel examine a red imported fire ant colony for the presence of ants infected with the recently discovered yellow-head disease.

An ARS-funded areawide project began in March 2001 to demonstrate how to keep fire ant populations at very low levels by combining strategic pesticide applications with phorid flies and *T. solenopsae.* Diverse demonstration sites—as large as 300 acres—in five states were chosen for the project to represent the fire ant's infestation range, according to Williams. ARS is coordinating the major activities of four land-grant universities and other organizations associated with the project for 4 to 5 years.—By **Jim Core,** ARS.

This research is part of Arthopod Pests of Animals and Humans, an ARS National Program (#104) described on the World Wide Web at http://www.nps.ars.usda.gov.

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Fungus Set To Fight Insect Pests

Trouble is brewing for silverleaf whiteflies and other plant pests.

The Agricultural Research Service (ARS) and Encore Technologies, LLC, are considering a licensing agreement to commercialize ARS' patented (No. 5,968,808) new method of mass-producing the fungus *Paecilomyces fumosoroseus* as a bioinsecticide.

Microbiologist Mark Jackson developed the method, which uses deep-tank liquid culture fermentation, based on his fungal nutrition studies at ARS' National Center for Agricultural Utilization Research, Peoria, Illinois. There, Jackson combined the method with an Encore procedure for formulating *Paecilomyces* spores into an air-dried powder that can be wetted and sprayed onto plants.

"I see this being used in greenhouses, out in the crop field, for organic uses, and the home pest control market," says David A. Goulet, Encore's president. "Anyplace where chemical use is a concern, this would fit the bill."

Thrips and aphids are just a couple of the pests that *Paecilomyces* could see action against.

Jackson has his sights on the silverleaf whitefly, a sap-sucking pest of 600 plant species, including cotton, tomatoes, and poinsettias. Whitefly infestations in these and other U.S. crops cause multimillion-dollar losses.

Paecilomyces kills whiteflies by snaking tiny filaments into their bodies to feed and grow. Jackson compares the sight to "*Gulliver's Travels,* when the Lilliputians tie down the giant." People and other animals face no such danger, but infected whiteflies die within several days. New spores then emerge to infect other whiteflies, sparing nonhost insects as they spread.

Despite such advantages over chemical controls, past attempts to formulate *Paecilomyces* have stumbled on high production costs and other problems, Jackson says. Only one U.S. company—Certis USA, LLC—has registered it as a biopesticide (Apoka Strain 97).

"The big problem with biocontrol agents is producing them cost-effectively and keeping them stable once produced," says Jackson. "We've overcome this problem by developing new technologies for spore production and stabilization." These involve feeding a *Paecilomyces* culture—any of 35 known strains—a diet of carbon, nitrogen, and other nutrients inside fermentation tanks. Combining the fermentation culture with appropriate temperature and aeration stimulates the fungus to produce highly infectious blastospores, which are ideal for bioinsecticide uses.

"We can do a 2-day fermentation run and obtain a yield of a billion blastospores per milliliter," Jackson reports. What's more, the blastospores can survive 1 to 2 years of cold storage.

Both features are expected to revive prospects for the fungus as a commercial bioinsecticide that can compete with, or complement, conventional chemical controls. In poinsettias, for example, the fungus might prove useful against whiteflies that threaten the crop 6 to 8 weeks before harvest, when insecticides aren't applied for fear of mottling the plants' coloring.

"Technically," says Goulet, "this material is 2 years from being ready to go" to market as a bioinsecticide product. First, though, it would require EPA registration.—By **Jan Suszkiw,** ARS.

This research is part of Crop Production, Product Value, and Safety, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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SCOTT BAUER (K10621-1)

At the ARS National Center for Agricultural Utilization Research, microbiologist Mark Jackson and technician Angela Payne evaluate the fermentation progress of the bioinsecticidal fungus *Paecilomyces fumosoroseus,* destined for use in field trials.

New Cuisine Wins Rave Reviews From Honey Bees

Honey bee hives from Maine to California are abuzz with news about a new food that originated from a chance conversation between two ARS scientists. At an Entomological Society of America meeting in the summer of 2001, entomologists Gloria DeGrandi-Hoffman, who specializes in honey bee research, and Allen C. Cohen, who is internationally recognized for his pioneering work with foods for insects, met and discussed a problem that has plagued California's almond growers for years.

These orchardists, producers of the nation's $1 billion almond crop, need bustling colonies of honey bees—alive with strong workers and healthy young, known as brood—to pollinate their vast orchards during the winter months. That's the time of year these trees begin to flower. But it's also when bees are in a near-hibernating state and not being very industrious.

Cohen and DeGrandi-Hoffman, now research leader at the ARS Carl Hayden Bee Research Center, Tuscon, Arizona, decided to collaborate in developing a recipe for an artificial diet that would give honey bees the whole package of nutrients they need in an easily digestible liquid. This would keep the bees rearing brood and ready to work once the almond blossoms appear.

Presently, beekeepers use soy patties to feed bees during winter, before almond blossoms—floral treasure troves of pollen and nectar—are readily available. The major problem with these patties is that they are costly and labor-intensive. Beekeepers must either go from hive to hive and manually insert the patties or place large tubs of the dry patty mix near their beehives.

The patties—typically made of corn syrup, soy flour, and brewer's yeast—create another problem. For reasons not yet understood, bees that eat soy patties eventually lose their ability to produce a food, called worker jelly, that's vital for the brood.

With a liquid diet, a machine already used by beekeepers would be able to easily pump the bee food into the hives, a far less labor-intensive approach, says Cohen. He recently retired from the ARS Biological Control and Mass Rearing Research Unit in Mississippi State, Mississippi. In the late 1980s, he was stationed at the Bee Research Center and worked with DeGrandi-Hoffman there.

Cohen started with an existing diet that he created for indoor rearing of lygus bugs. It was patented by USDA in 2000. To meet the needs of beekeepers, he converted the lygus bug food from solid to liquid, blending ingredients that provide vital nutrients.

Cohen wanted to create a formula that would combine the sweetness of nectar and the nutritional punch of pollen into a digestible, absorbable food that bees would gobble up.

Honey bees devour a new, nutrient-rich food. This artificial diet resulted from 5 months of research. Photo by Stephen Ausmus. (K10288-1)

Nectar is rich in carbohydrates. Pollen is packed with protein, vitamins, minerals, fats, and perhaps as-yet-undiscovered compounds essential for bees' survival.

"What we didn't know is whether bees would be able to take a liquid that combined the qualities of both nectar and pollen and process it in such a way that the formula would be completely nutritious for them," says Cohen. "That was really one of the most exciting possibilities—to see whether we could essentially fool the bees' natural digestive mechanisms."

STEPHEN AUSMUS (K10285-1)

Entomologists Gloria DeGrandi-Hoffman (right) and Gordon Wardell observe comparison feeding trials in which different diets are offered and bee preference is monitored.

Cohen's classic recipe, modified slightly, thus became the Arizona lab's starting point for a new menu option for domesticated honey bees, *Apis mellifera*.

With Cohen's formula in hand, DeGrandi-Hoffman and Gordon I. Wardell, an entomologist and corporate collaborator at Tucson, mixed batches of the creamy-white liquid for bees to taste-test.

The intent was to create an elixir so delectable that adult bees would not only eagerly eat it but would also, as is their usual practice, store some of it in the hive for nurse bees—the hive's round-the-clock nannies—to feed to the colony's brood.

For the investigations, Wardell assembled 12 small hives, then enclosed them with netting so bees couldn't sneak out during the tests to bring their familiar foods back to the hive. Each hive housed about 3,500 to 4,000 bees. "We offered the bees one kind of food—either sugar-water, pollen, or the new liquid, which we poured into petri dishes," says Wardell. The bees ate little, if any, of the new concoction.

STEPHEN AUSMUS (K10266-1)

Gordon Wardell prepares a variation of Allen Cohen's classic recipe, a scrumptious blend of vital nutrients that finicky bees just can't resist.

To make it more appetizing, the researchers launched a series of new experiments, presenting variations of the baseline recipe for the bees' approval. The scientists structured these experiments so that despite natural variations from one six-legged taste-tester to the next, the findings would be statistically sound.

After 5 months and nearly 80 reformulations, the team hit on an apparently scrumptious creation that the finicky bees just couldn't resist. The scientists then tested it in full-size hives, each accommodating about 30,000 bees. Wardell and co-worker Fabiana Ahumada-Segura fitted each hive with a small window so that they could spy on the dining bees.

"At one point," says Wardell, "we saw 75 to 100 bees jammed around a petri dish, pushing and shoving each other to get to the food. In the commotion, some of the bees fell into the petri dish. It was like a bee mud-wrestling match."

Adds Cohen, "This is somewhat historic because people have tried for the past 50 years to develop diets for bees but were unable to get the bees to continue to rear brood on those formulas. With the new diet, bees have been able to rear continuous generations of brood."

To see whether the new culinary offering would please bees elsewhere, Wardell sent samples to commercial beekeepers in Arizona, California, Georgia, Maine, Michigan, New Mexico, and Pennsylvania. "All the beekeepers reported that their bees liked this recipe," Wardell says. "One beekeeper asked an assistant to take a ruler out to a hive and measure how much food was left after the bees had about 4 hours to try it out. To their amazement, all the food was gone."

More work is needed, however, before the experimental food is ready for commercial and hobbyist beekeepers to use. "We want to cut the cost so the food is affordable," Wardell explains. "We're using very pure, pricey ingredients, but we intend to substitute less-expensive compounds." The scientists also aim to develop a dry mix. Lighter than the liquid, it should be cheaper to ship.

Further fine-tuning of the formula may boost brood survival rates and lengthen adult bees' typical 4-week life-span. Currently, both these measures are about the same among bees reared on pollen patties and those fed the novel food.

Another planned upgrade: compounds known as feeding stimulants that may entice bees to eat even more. Though these are already standard ingredients in other foods formulated for nourishing beneficial insects, apparently no one yet knows which feeding stimulants would appeal to honey bees.

The researchers intend to seek a patent for their bee cuisine.—By **Alfredo Flores** and **Marcia Wood,** ARS.

This research is part of Crop Protection and Quarantine (#304) and Crop Production (#305), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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Zapping Sweetpotato Weevils

Irradiation has yet another useful application.

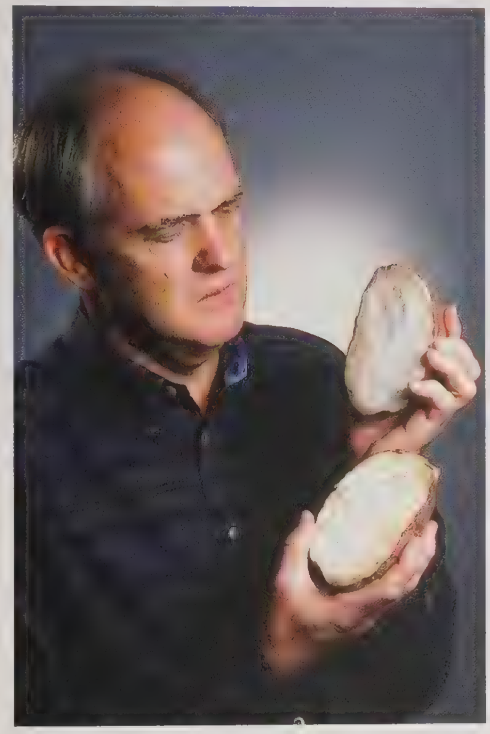
In the United States, the sweetpotato is best known as a holiday treat that goes with turkey or ham when families gather for Thanksgiving. What many Americans may not know is that the versatile vegetable is the world's fifth-most-produced food crop, with more than 133 million metric tons harvested worldwide every year. That translates to about 47 pounds for every person on the planet, though we in the United States consume only 4 pounds each, on average.

But the popular sweetpotato is threatened by the sweetpotato weevil, *Cylas formicarius elegantulus*. Although the pest is widely distributed throughout sweetpotato-growing regions of the world, areas that do not have it—such as the southwestern United States, around the Mediterranean, and parts of Latin America and Africa—understandably quarantine against it, to prevent its spread.

The weevils cause serious damage by laying their eggs at the base of plants in the field. The larvae that hatch burrow into the roots, causing them to rot. Among food plants, *C. formicarius* attacks only sweetpotatoes and continues to damage roots after they have been harvested and put in storage.

Gassing with methyl bromide has been the only method to control sweetpotato weevils on market-bound roots, but it usually damages them. Furthermore, methyl bromide is being phased out for most uses because it is thought to damage the stratospheric ozone layer that provides protection from ultraviolet light.

SCOTT BAUER (K10125-1)



Entomologist Guy Hallman examines white-fleshed sweetpotatoes for reaction to irradiation quarantine treatment against sweetpotato weevil.

From the spring of 1999 to April 2000, Guy Hallman, an entomologist at ARS' Kika de la Garza Subtropical Agricultural Research Center in Weslaco, Texas, studied irradiation as a postharvest treatment to meet quarantine regulations for interstate shipment of roots that can harbor sweetpotato weevils. Exposure sterilizes—but doesn't kill—them. The insects may remain on the roots until they die in a few weeks, but they can't reproduce and they do only negligible damage.

To prevent the spread of weevils, irradiation is being used by growers in southern Florida to treat boniato-type sweetpotatoes shipped out of state. These white-fleshed sweetpotatoes are popular with Caribbean and other immigrants living in the United States and also represent a valuable export.

Hallman previously worked with irradiation to curb fruit flies, but he says that his work on this weevil stands out. "It's the first time irradiation is being used as a quarantine treatment against an adult insect," he says. "That's significant. Because the insects aren't killed, inspectors must have complete confidence in the treatment. Otherwise, finding live adults would be cause for rejecting a shipment."

In 2000, the first year the technique was used to treat boniatos, 175 metric tons were irradiated and shipped. By 2001, the total had grown to 189 metric tons, and it's expected to reach 200 metric tons by the end of 2002. Irradiation has the potential to treat a significant share of the world's sweetpotatoes.—By **Alfredo Flores,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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PEGGY GREB (K10575-1)

Operation Full Stop 2003

Stopping

Part of a Formosan subterranean termite nest showing the extensive system of galleries the insects create. The nest was found in an apartment wall in the New Orleans French Quarter.

PEGGY GREB (K10571-1)

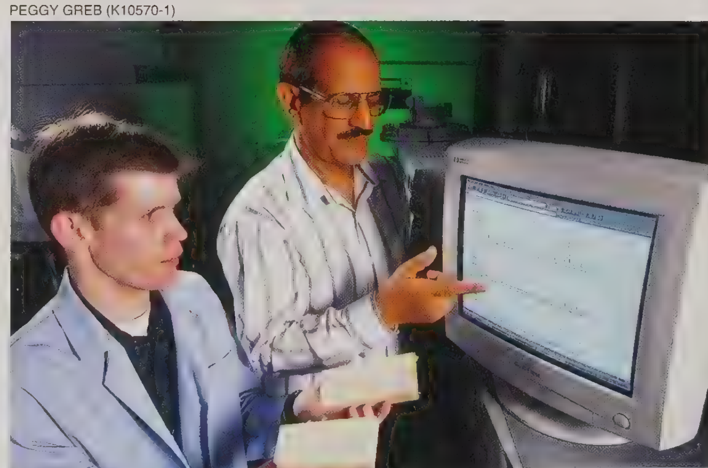
Formosan subterranean termites feeding on spruce (left) and birch wood blocks.

In New Orleans, it starts during the twilight hours in late April. Thousands of tiny, winged termites—alates—begin swarming lampposts. In some areas, the air becomes so thick with the flying pests that streetlights dim behind hovering brown clouds. As the alates mate and drop their wings, the street crackles underfoot.

New Orleans, Louisiana, can reasonably be called the termite capital of the continental United States. It has been overrun by *Coptotermes formosanus*, the Formosan subterranean termite. Experts believe that the stowaway species entered the country on ships sailing back from the Pacific after World War II. Since their arrival, these termites have multiplied exponentially in the moist, warm, southern Louisiana climate. They've also established themselves in 10 other states and cost U.S. consumers $1 billion every year in control costs and damages.

In 1998, the Agricultural Research Service, with several major cooperators, launched a national campaign to reduce the population of these voracious invaders and lower their cost to society. Now, the researchers are taking stock of the progress they've made and focusing on new endeavors.

PEGGY GREB (K10570-1)



In studies to determine whether changes in diet trigger termite aggression, technician Christopher Florane (left) and entomologist Ashok Raina discuss gas chromatography-mass spectrometry profiles of termites fed different types of wood.

No Quarter for Termites in the Vieux Carré

New Orleans' historic French Quarter is the epicenter of the Formosan infestation—a worst-case scenario come to life. Many buildings in this part of the city

the Swarm

contain wood that dates back a century or more. And the city itself gets about 60 inches of precipitation annually. With an abundant food supply and a ready source of moisture, Formosan colonies have been able to increase and expand here over the years.

Exacerbating the problem? Construction practices and a defensive, protect-the-structure approach to termite control made traditional chemical treatment difficult. So near-perfect conditions for termite colonies, combined with limited effective pesticides, contributed to rapid population growth in the late 1980s.

By the mid-1990s, the U.S. Congress saw that without some type of intervention to contain the Formosan scourge, many properties within the world-famous Vieux Carré would be reduced to the lacy grillwork they're so famous for. Now, 5 years after Operation Full Stop began, the situation doesn't seem nearly as dire. It looks as though Formosan populations can be managed. The program's coordinators stress one of the most critical lessons they have learned from their research: the importance of having an offensive, areawide management approach.

"Areawide management is key," says Alan Lax, leader of ARS' Formosan Subterranean Termite Research Unit at the Southern Regional Research Center (SRRC) in New Orleans. "We've been able to reduce termite numbers in the French Quarter because we're treating entire blocks, not just individual homes or businesses."

Frank Guillot, ARS' Formosan termite program coordinator, concurs. "We've been keeping track of alate numbers inside and outside the treatment zone since 1998, the year we began the program. In 1998, about 70 percent as many alates were captured inside the treatment zone as were captured outside. By 2002, that number had been reduced to 42 percent." Visual inspection of easily accessible areas of structures in the French Quarter since the beginning of the program indicated no evidence of newly active termites or the damage they can cause. But advanced technologies—infrared, acoustic, and motion-detection devices—are now being used to search for termites or their damage in areas not readily accessible.

Dennis Ring, an entomologist with the Louisiana State University Agricultural Center (LSU AgCenter) and principal investigator of the French Quarter program, explains that instead of applying repellent chemical barriers around buildings, participants are having baits or nonrepellent liquid termiticides installed around their properties. They're "playing offense" against the termites instead of defense, and it's working.

Encouraged by the program's success, researchers expanded the original 15-block treatment zone in 2002. Ring says, "Public participation in the original area is nearly 100 percent. We've moved out one block in all directions, so now we're covering close to 30 blocks. We've already got 60 percent of the properties in the expanded area into the program, and people continue to join up."

Says Lax, "Before Operation Full Stop began, there was this pervasive myth that you shouldn't pay for commercial termite treatments because they don't work. In fact, only 15 to 20 percent of the properties in the French Quarter had undergone any type of termite prevention program.

"We've shown property owners that termite treatments can be effective under the right conditions. Our latest estimate is that even outside our treatment zones, where homeowners receive no help from the program, 40 percent of the properties have undergone some type of treatment."

High-Tech Detection

Having access to highly sensitive instruments that can pinpoint exactly

PEGGY GREB (K10580-1)

Formosan termites can infest railroad ties like this one. Termite Board entomologist Janet McAllister inspects a tie for termites and nests in New Orleans.

PEGGY GREB (K10565-1)

Left: Cottony mold, *Paecilomyces fumosoroseus*, is proving very effective as a natural termite-control agent. ARS microbiologists Mark Jackson and Maureen Wright observe *Paecilomyces* cultures before exposing termites to the fungus.

Right: ARS technician Pete Levy (left) and LSU Agricultural Center cooperator Chris Morel inspect one of many in-ground stations placed throughout the New Orleans French Quarter to monitor the presence and activity of Formosan subterranean termites.

PEGGY GREB (K10585-1)

where the termites are hiding is an important aspect of the offensive strategy. Jack Leonard of the New Orleans Mosquito and Termite Control Board (NOMTCB) has been using one such instrument in the field for several years. It's a thermal imager that can detect termites behind walls and in other hard-to-reach places. The late Bob Melia, who owned Real Time Thermal Imaging, helped develop the application. He died in 2001, but his family has continued running his business. Leonard uses the technology to evaluate different termite control projects sponsored by Operation Full Stop. Most recently, he checked schools in the Orleans Parish where control treatments took place.

"The thermal imager is a handheld device about the size of an 8-mm camcorder," explains Leonard. "It takes practice and experience in the field to read the black-and-white images, but we've had good success in detecting infestations. We hope to train more people to use it."

SRRC entomologist Weste Osbrink and Richard Mankin, an entomologist at ARS' Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida, have been focusing on a different type of technology to uncover termite infestations—acoustics. Osbrink is a termite expert, and Mankin is an expert at detecting insects by their sounds.

"We worked together to create a device that detects termites through the sounds they make as they're feeding on wood," says Osbrink. Although such devices are not yet in widespread use, they have great potential to help scientists and pest control operators manage the termite problem.

Additional research is being conducted by cooperators at the National Center for Physical Acoustics in Oxford, Mississippi, to develop technology and systems to detect termites or their structural damage.

Termite-ators: They'll Be Back?

Researchers involved in Operation Full Stop's Armstrong Park project have helped prove how important areawide management and constant vigilance are. Nan-Yao Su, an entomology professor at the University of Florida, and Matt Messenger, an entomologist with NOMTCB, lead the project.

New Orleans' Louis Armstrong Park covers about 31 acres and sits next to the north side of the French Quarter. Su and Messenger, with their cooperators, have been studying the foraging behavior of the park's 4 to 6 native subterranean termite colonies and 14 to 18 Formosan subterranean termite colonies. In one study, they determined how quickly other termites would reinvade an area after a single colony had been eliminated. Their work showed that if untreated, both Formosan and native colonies will begin to occupy vacated territory within days. Complete reoccupation by Formosan colonies may take as little as 6 months.

The scientists have just begun a new project. Su explains, "We're trying to eliminate all the termites in Armstrong Park so that we can see where new colonies will take hold. This will help us better understand the ecological succession of termite infestations. It will also help us focus on where future problems may occur in an areawide management approach."

Prime Target: Termites' Tastebuds

One of the most effective ways of getting rid of a termite colony is with a toxic bait matrix—a combination of materials that the pests like to eat and a slow-acting toxic agent that ultimately kills them. SRRC entomologists Juan Morales-Ramos and Guadalupe Rojas are experts on termite nutritional preferences, and they have created several matrix formulations.

Rojas says, "In the lab, we have hundreds of petri dishes filled with termites, and we use the termites like sensory panelists. We feed them different nutrient mixtures to find out what they like to eat best."

PEGGY GREB (K10587-1)

Left: Underground monitoring stations are installed in New Orleans' Louis Armstrong Park. Aaron Mullins, an entomologist with the New Orleans Mosquito and Termite Control Board, checks for termite activity in the monitors.

Right: In spring, alate traps are placed on lampposts throughout the French Quarter to monitor mating Formosan subterranean termites. Chris Morel (left) and student worker David Cox inspect an alate trap.

PEGGY GREB (K10589-1)

In October 2002, Rojas and Morales-Ramos completed a 2-year, areawide, bait matrix study in residential Mississippi neighborhoods in Poplarville, Picayune, and Biloxi, at Keesler Air Force Base. The research was done with entomologist David Nimocks, president of Ensystex of Fayetteville, North Carolina. The scientists have a cooperative research and development agreement with Ensystex, and ARS has granted an exclusive license to the company to manufacture the bait matrix. Other collaborators included scientists from Mississippi State University's Coastal Research and Extension Center, including David Veal, head of the center; researchers Christine Coker and Patricia Knight; and research associates Margaret Lockwood and Larry Etheridge.

Mississippi has a growing Formosan termite problem, although populations there aren't nearly as dense as they are in New Orleans. Using an ARS bait matrix containing chlorfluazuron, ARS and Ensystex researchers were able to reduce native and Formosan termite activity by 95 percent in one test area in Picayune. In a related study, they found that one of their bait matrices, which incorporates diflubenzuron, was also effective against red imported fire ants. These pests must be controlled in and around bait sites because their presence repels the termites. And fire ants are another major urban pest. Having filed a patent application on the ant bait matrix, the scientists are currently working with Ligia Hernandez, product development manager for Waterbury Co., Inc., Independence, Louisiana, which has applied for a license for its manufacture.

Rojas says that they have started new experiments in cooperation with MSU, Ensystex, Waterbury, and James M. Spiers and Formosan termite personnel at ARS' Small Fruits Research Unit in Poplarville. The goal is to focus even more attention on integrated methods to best control both termites and ants.

Termites vs. Essential Oils and Mold

By studying different plant extracts, entomologist Gregg Henderson at the LSU AgCenter has found compounds that are effective against termites and other insects, including red fire ants, mosquitoes, ticks, and cockroaches. One such compound, nootkatone, is derived from essential oils in vetiver grass.

Recently, Henderson and his collaborators have been evaluating how well compounds such as nootkatone work to protect wood from Formosan termites. Results have been promising. Henderson says the compounds seem to affect termites' neurotransmitters and stop their movements. If further experiments prove successful, these essential oils could replace chemicals such as copper, chromium, and arsenic currently used to treat lumber against insect attack.

At ARS' Natural Products Research Unit in Oxford, Mississippi, chemists are extracting natural compounds from other sources, seeking new active ingredients that could act as termiticides or form the structural basis for developing such ingredients.

SRRC microbiologist Maureen Wright has been having good success with a natural termite control agent. It's derived from a cottony mold called *Paecilomyces fumosoroseus*. Wright heard about its biological control abilities from Mark Jackson, a microbiologist with ARS' National Center for Agricultural Utilization Research, in Peoria, Illinois.

Jackson had developed an efficient and relatively inexpensive way to produce large amounts of the fungus to control whiteflies, aphids, and other agricultural pests. Wright tested samples of the mold against Formosan termites and came away with impressive results: It killed 100 percent of the termites in less than a week.

Wright has demonstrated that termites exposed to the mold transfer it to other

members of their colony. She's also shown that it can be effective in various settings. Retired SRRC chemist Bill Connick helped develop several application formulations. A termite-infested tree, for instance, might require a different application method than a wooden structure. ARS is currently negotiating with a company that wishes to license the technology.

What Makes Termites Tick

Besides developing new methods of termite control, Operation Full Stop scientists have also been learning more about the Formosan termite's basic biology. Entomologist Ashok Raina leads a group within the main termite research unit at SRRC. His group observed and recorded, for the first time, mating in the Formosan termite. They also discovered a contact sex pheromone in the female's tergal gland—the first time such a pheromone has been identified for any termite species.

Recently, Christopher Florane, a University of New Orleans graduate student who has been working with Raina's group, discovered new information about why termite colonies attack each other. He found that the basis of aggression was the type of wood eaten by the termites. Only when two termite colonies feed on two different types of trees do they become aggressive toward each other. Florane's abstract and presentation about his finding won a President's Prize at the Entomological Society of America's national meeting in Fort Lauderdale, Florida, in November 2002. He is currently working with Raina to determine exactly how changes in diet trigger aggression.

Termite Research Chugs Along

Also in 2002 . . . NOMTCB entomologist Janet McAllister began a new study—in cooperation with Dow AgroSciences—that may eventually help the New Orleans termite control zone grow still larger. She and her collaborators are trying to find the best way to treat railroad ties that could be serving as huge Formosan termite reservoirs.

PEGGY GREB (K10582-1)

Termites living in railroad ties could reinfest treated areas of New Orleans. NOMTCB termite inspectors Gustavo Ramirez (foreground) and John Stennett remove monitoring devices to count termites and score wood consumption.

McAllister explains, "There are two railroad lines running next to the French Quarter and the Mississippi River that fall outside the current Operation Full Stop treatment zone. The railroad ties are treated with creosote, but where the ties have cracked, termites have invaded untreated pockets of wood inside."

The termites that live in the wooden ties might eventually move right back into the areas where other Formosan termite populations have been reduced. McAllister and her team are focusing on a little less than a mile of track and have already counted eight separate colonies in the study area. They plan to test different types of baits and toxins, placing them at varying intervals along the tracks to see which configuration works best. She expects the project to last several years.

Edgar G. King, Jr., ARS' Mid South Area director, who's been with Operation Full Stop from the beginning, says, "I am pleased with all the cooperation we've received from our partners in the program. Their contributions have been critical to successes in the Vieux Carré, Armstrong Park, and south Mississippi. Fundamental research on the termite's biology and ecology, development of new technologies like the bait matrices and detection devices, and discovery of new chemical and biological control agents will all help ensure sustained control of this destructive pest. Our goal now is to implement these measures throughout larger areas and eliminate the Formosan subterranean termite as a pest of dwellings in these treated areas."—By **Amy Spillman,** formerly with ARS.

This research is part of Arthropod Pests of Animals and Humans, an ARS National Program (#104) described on the World Wide Web at www.nps.ars.usda.gov.

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STEPHEN AUSMUS (K10126-1)

Entomologist Jim Hansen infests apples with codling moth larvae while technicians Millie Heidt (left) and Michele Watkins (center) remove larvae from a rearing media.

Radio Frequency Puts the Heat on Plant Pests

To prevent influx of pests that could create agricultural problems, produce-importing nations enforce strict rules—depending on the commodity and the infesting insect. The rules often require vulnerable produce to be treated in some way that ensures destruction of pests.

For several decades, methyl bromide has been a mainstay treatment to kill a wide array of quarantined pests as well as those encountered in orchards, packinghouses, and food plants. But this potent chemical fumigant is being phased out because of evidence linking it to damage to Earth's ozone layer.

Although the effectiveness of using radio waves to kill destructive insects in agricultural products has been known for 70 years, the technique has never been applied on a commercial scale. A recent cooperative effort by four ARS research laboratories and two universities aims to overcome the technical barriers for the use of radio wave heating to control pests on a commercial scale.

Electromagnetic waves of radio frequency can make molecules vibrate and heat up—like microwaves heat food. The trick is to kill pest insects without killing the taste or texture of the food they infest.

Since 2000, a team led by Juming "Jimmy" Tang of Washington State University (WSU) in Pullman, involving four ARS laboratories and the University of California-Davis (UC-Davis), has been working on a 4-year study to see whether radio waves would be an economical, environmentally friendly alternative to methyl bromide and other chemicals to effectively rid fruits and nuts of live, quarantined insects.

In Texas—*It's Chiefly Citrus*

In Weslaco, Texas, ARS entomologist Guy J. Hallman is checking out use of radio frequency treatment of citrus against the Mexican fruit fly. He's in the Crop Quality and Fruit Insect Research Unit at the Kika de la Garza Subtropical Agricultural Research Center. Hallman is developing a device to simulate what's needed to heat-treat citrus fruit with radio waves commercially.

"We're trying to bridge the gap between the laboratory and real world," says Hallman. "Once we know how to treat fruit in a commercial situation and how much it will cost, any producer, shipper, or packinghouse operator can use the information to decide whether radio wave pest control is a viable option."

In Hallman's system, citrus fruit would pass through a conveyor between a series of radio frequency heaters. To simulate a commercial system in the laboratory, the fruit are conveyed in a circulating water bath to keep them moving during heating. This would prevent the fruit's overheating from extended contact with any one area of the bath. And to ensure continuous heating from the peel in to the fruit's center—essential to killing all fruit flies that might be present—a bumper would dunk any fruit that bobbed above the

water surface. This prevents dark-black rings from forming around the fruit at the water's surface because of an energy concentration where the water meets the air.

A method using just hot air to treat fruit in boxes or bins has been tried commercially in Mexico with mixed results. It takes hours to complete and puts a strain on the fruit's skin, sometimes causing heat damage. Radio frequency heating can be done in less than half an hour and is less damaging, since the fruit is heated uniformly throughout.

Hallman has focused on grapefruits but is also working with other citrus, including oranges and tangerines. The larger the fruit, he notes, the harder it is to heat uniformly and the more likely to form hot and cold spots.

"This multi-lab project is making a serious effort to take a look at things that haven't been looked at with radio frequency heating," says Hallman. "I think we stand a good chance of finding out how radio frequency disinfestation can be done to a large volume of fruits or nuts—and if it can be done on a commercial scale." He predicts that by this summer he'll have a good idea of what the treatment will cost.

In Washington—*Apples and Cherries*

If you're in Wapato, Washington, don't be enticed by the apples floating in the tub in James Hansen's laboratory. You don't want to go bobbing for them.

There are several reasons, says Hansen, an entomologist with ARS' Yakima Agricultural Research Laboratory. The first is that this particular tub is filled with salt water. Second, if you were to latch onto one of the apples with your teeth, you might bite the proverbial worm—a larva hatched from a codling moth egg.

And Hansen wants these apples intact—no tooth marks, please. Such a mark might skew the results of tests he is conducting on use of radio waves to rid the fruit of

SCOTT BAUER (K10213-1)

Technician Carlos Solis, of the ARS Kika de la Garza Subtropical Agricultural Research Center, in Weslaco, Texas, places oranges in a water bath heated with radio waves to kill fruit flies. The water is circulated to heat the oranges uniformly and prevent fruit damage.

live, pesky insects like the codling moth before market—or shipment to trading partners like South Korea and Japan, where such pests might not already occur. Japan is particularly stringent about what phytosanitary methods it will accept for disinfesting fresh produce.

This rule also applies to sweet cherries, a tree fruit commodity that generates over $145 million in yearly national export sales, notes Hansen. In cooperation with the team led by Tang, a professor in biological systems engineering at WSU, Hansen plans to "bathe" tubs full of apples and cherries with radio waves to determine exposure times that will kill codling moth larvae without affecting fruit quality.

From two lines of research—one focusing on the insects, the other on fruit quality—and three major disciplines—engineering, entomology, and plant physiology—the collaborators hope to position radio wave treatment as a technology that can be readily adopted by commercial packinghouses or quarantine operations in lieu of methyl bromide.

"Without suitable alternatives to methyl bromide, we're going to be up a creek," ARS horticulturist Stephen R. Drake says of the U.S. fruit industry's fight against quarantined pests. He is with ARS' Tree Fruit Research Laboratory in Wenatchee, Washington.

"Ideally, you want to treat the fruit with radio frequency while it's being packed and designated for a particular market," Hansen explains. "Commercial packers can't afford to have produce sitting there, so we want this treatment to kill the insects as soon as it can."

Tang, Hansen, Drake, and Lisa Nevens, an ARS entomologist at Yakima, first began working on the radio frequency project in 1996.

In California—*Tree Nuts and Dried Fruits*

Meanwhile, in California, entomologist Judy A. Johnson is also keenly attuned to the potential of radio frequency energy to zap destructive insects. Her primary targets? The wiggly larvae of the navel orangeworm, Indianmeal moth, and codling moth. These insects are among the worst enemies of walnuts, almonds, and pistachios and of dried fruits such as figs and raisins. Johnson has newly added red flour beetle to her list of culprits—a lesser pest of the nut and fruit crops but a major problem in flour mills and food-processing plants.

Johnson is doing the radio frequency work at the ARS San Joaquin Valley Agricultural Sciences Center at Parlier, near Fresno, in close association with cooperators at WSU and UC-Davis. They have already developed a preliminary picture of the target insects' ability to endure heat—their "thermal tolerance."

The laboratory experiments that Johnson and her ARS and university colleagues conducted are the first to

extensively detail the thermal tolerance of the navel orangeworm, Indianmeal moth, and codling moth.

For one test, Johnson and co-investigators drilled tiny holes in over 500 in-the-shell walnuts; enticed the slender, whitish navel orangeworms to enter the shells; then plugged the holes to block the insects' escape. The scientists then tried some novel combinations of radio waves and hot forced air, that is, air that's heated and blown into the test chamber holding the nuts. Radio waves, alone or combined with hot forced air, were used to heat the nuts to 55°C (131°F) in about 5 minutes. Hot forced air was then used to keep the nuts at 55°C for 5 or 10 minutes.

"All the treatments killed 100 percent of the navel orangeworms," reports Johnson. What's more, tests led by co-researchers Tang at Pullman and Elizabeth J. Mitcham at UC-Davis, showed that the treatments didn't harm the quality of the nuts—even in long-term storage.

"That's critical," Johnson points out, "because walnuts are often stored for a year or more before they show up at your supermarket."

In addition, walnuts are rich in oil, so they're more vulnerable to heat damage than some other kinds of nuts, such as almonds. "That's why we think that if a treatment is okay for walnuts," Johnson continues, "it will likely be okay for almonds."

Indianmeal moths proved to be more susceptible to heat than navel orangeworms are. "Indianmeal moths are silvery, and wedge-shaped. If you open a kitchen cupboard where you keep raisins or breakfast cereals and a little moth flies out at you," Johnson explains, "chances are it's an Indianmeal moth."

For this experiment, Johnson and her colleagues used metal heating blocks, or plates, specially designed by Tang for the radio frequency research. They placed target insects in the small gap between the plates, which were then heated to specific temperatures and held at those temperatures for precise periods.

She and her associates assembled some 15,000 Indianmeal moth larvae for the study, exposing them to temperatures ranging from 44°C to 52°C (about 111 °F to 126°F) for 2 to 100 minutes. "In general," Johnson comments, "the lower the temperature, the longer it took to kill the larvae. These readings are the basis for a new, math-based model for projecting the rate of kill at other time-and-temperature regimens. Because of the large number of larvae tested and the range of temperatures that we investigated, we're very confident of the accuracy of the model's projections."

Johnson and her cooperators also used the special heat plates to discover more about the amount of time and heat that it takes to kill navel orangeworms. They exposed 15,000 navel orangeworms to temperatures ranging from 46°C to 54°C (about 115°F to 129°F) for 1 to 120 minutes. Using the new data, they created a mathematical model of the navel orangeworm's thermal tolerances.

The findings from the Texas, Washington, and California experiments are an essential starting point for making the radio frequency energy approach a success, Johnson says. The scientists have published their findings in the *Journal of Economic Entomology*, *Journal of Stored Products Research*, and *Postharvest Biology and Technology*.—By **Alfredo Flores, Jan Suszkiw,** and **Marcia Wood,** ARS.

This research is part of Methyl Bromide Alternatives, an ARS National Program (#308) described on the World Wide Web at http://www.nps.ars.usda.gov.

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STEPHEN AUSMUS (K10218-1)

Technician Michele Watkins infests an apple with codling moth larvae. The apples will then be subjected to radio waves to determine exposure times that will kill the larvae without affecting fruit quality.

Monitoring System Counts Insects, Identifies Species

Look out, grain-munching insects! A new, enhanced version of the world's first automated insect-monitoring system recently hit the market—and it's looking for you.

Developed by ARS and patented in 1997, the original Electronic Grain Probe Insect Counter (EGPIC) was a major improvement over other grain probe traps used to detect insect infestations in grain bins. It used infrared beam sensors to quickly and accurately count insects and record the time of day as they dropped through the probe trap. Grain managers no longer had to enter bins and collect grain samples or empty traps to determine insect population densities.

Dennis Shuman, an electrical engineer in the Postharvest and Bioregulation Research Unit, Center for Medical, Agricultural, and Veterinary Entomology, in Gainesville, Florida, invented EGPIC with ARS colleagues. He says the newest version of EGPIC, now named "StorMax Insector," helps managers make even better decisions when monitoring infestations.

Developed through a cooperative research and development agreement with a Canadian grain-management company called OPI Systems, Inc., StorMax Insector is now commercially available. The easy-to-use system allows companies to avoid or reduce use of insecticides by using them or nontoxic alternatives only when indicated by monitoring, rather than scheduling preventive fumigations.

STEPHEN AUSMUS (K10502-3)

In a test of grain inside mini-silos, electrical engineer Dennis Shuman removes an Insector probe receptacle. He'll count the insects caught in the receptacle to determine the probe's accuracy.

Catch Them As They Fall

Depending on their geographical location, grain managers may only be concerned with about a dozen insect species. A previous EGPIC revision identified insects by size and shape. As an insect fell into the system's trap and interrupted the infrared beam, EGPIC registered its body before it plunged through a funnel and into a receptacle. The amount of light blocked from the beam determined how large a signal was generated. The system interpreted this signal to identify the insect's species. But the accuracy of this feature depended on the insect's position, so Shuman got the idea to put in a second beam—at a right angle to the first.

This additional beam provides a second viewing perspective. A microcontroller chip built directly into the new system's probe analyzes the two infrared beams' signals to determine the insect's species. It also reports the time of activity and the current temperature. These details give valuable insight into the nature and extent of an infestation.

"Insect behavior is affected by many factors," Shuman says. "For example, if it's cooler, insects don't move as much. We want to estimate the size of a given population in the grain bin by the number of electronic counts caused by insects entering the probes. Knowing the temperature and identifying the species is essential to making this calculation."

You Found Bugs. Now What?

There are several control options available to storage managers to keep insect populations at acceptable levels. These methods are sometimes combined, a strategy known as integrated pest management. One common method used by managers is called aeration. Aerating grain with fans reduces grain temperature, which slows insect activity. Another control is phosphine, the main fumigant used in the United States to treat bulk-stored grains. But there are concerns about phosphine's toxicity, and some evidence suggests that insects are acquiring resistance to the fumigant.

Shuman says the purpose of the Insector is to monitor insect infestations, not to eliminate them. For managers to apply the right treatment, they first have to know where and when they have a problem.

"Knowing the species helps managers use incoming insect counts—combined with knowledge of the individual species'

Right: **Cross section of the Insector probe showing the holes into which insects crawl and then fall into the receptacle.**

STEPHEN AUSMUS (K10505-19)

behavior and damage potential—to make control decisions," Shuman says.

An Insector probe is vertically inserted into bulk-stored grain. Insects find their way to the probe and climb one of many slanted, ramplike openings that were designed to keep grains from inadvertently entering and interfering with the count. Hundreds of probes can be used in the system, sending data from different sites to a handheld monitor or to the manager's office-based desktop computer. The recommended number of probes in each bin depends on its diameter as well as other factors.

The system comes with two receptacle options. One receptacle holds the insects until a manager collects them; the other has holes near the bottom where the insects are released. The percentage of insects collected is only a representation of the larger population present in a bin. Shuman says managers might prefer to use the collection receptacle initially to verify that the system is accurate. After that, they might choose to use the receptacle with the holes, which requires less maintenance and fewer trips to the bin to collect trapped insects.

Monitoring Success

Shuman established the EGPIC Working Group to further validate the performance of his invention. ARS entomologist James Throne at the Grain Marketing and Production Research Center in Manhattan, Kansas, is working with Shuman to coordinate large-scale EGPIC/Insector field tests with researchers from around the world to gauge its performance in different commodities and different environmental conditions.

Scientists at Montana and Oklahoma State Universities and Purdue University are validating the Insector's performance in commercial facilities that store wheat and corn. Shuman is also collaborating with ARS entomologist Paul Flinn at the Grain Marketing and Production Research Center to integrate the Insector system with ARS' Stored Grain Advisor (SGA). That system interprets information from standard sampling procedures to aid the storage manager in making pest management decisions in stored wheat. (See "Computer Figures Stored-Grain Insect Risk," *Agricultural Research,* June 1995, p. 22.)

Flinn is modifying the SGA software so it can automatically read Insector insect count data, interpret it, and then make recommendations about pest management to the storage manager.

Shuman envisions that commercial Insector systems will be installed in grain bins and elevators, data will be sent from the probes to computers in storage managers' offices, and the SGA program will automatically notify the storage manager when action is required.

This technology will benefit the U.S. grain industry and improve the quality of U.S. grain by lowering pest numbers, pest control costs, and pesticide residues. It will also improve safety at grain storage facilities by reducing the need for workers to enter the bins.—By **Jim Core,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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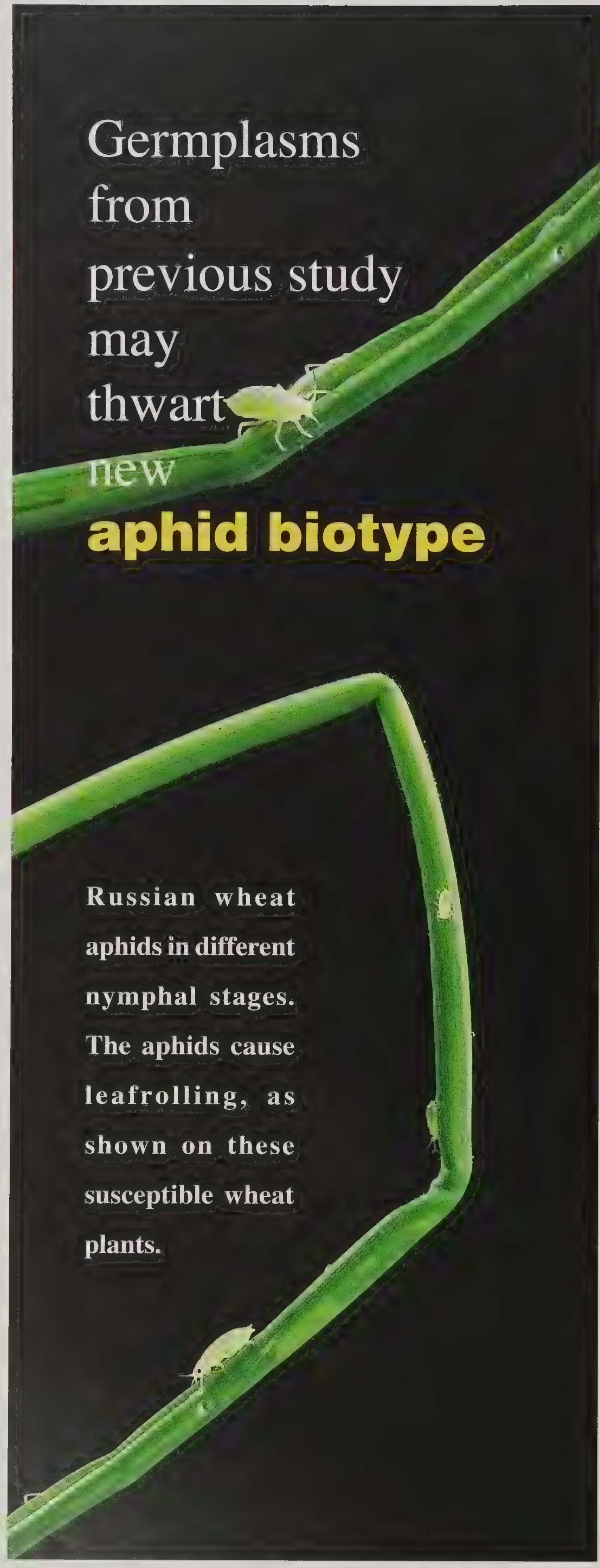
PEGGY GREB (K11167-1)



Germplasms from previous study may thwart new **aphid biotype**

Russian wheat aphids in different nymphal stages. The aphids cause leafrolling, as shown on these susceptible wheat plants.

Scientists in ARS's Wheat, Peanut, and Other Field Crops Research Unit have played a major role in controlling Russian wheat aphid before. Now, it seems this group from Stillwater, Oklahoma, may have done it again.

Starting in the late 1980s, entomologist James Webster (now retired) spent a decade sorting through thousands of wheat and barley germplasm samples, seeking accessions that showed resistance to the aphid, *Diuraphis noxia*. Geneticists Cheryl Baker and Dolores Mornhinweg then used these accessions to develop germplasm breeding lines that resisted the pest. These breeding lines led to aphid-resistant wheats, some of which have already been released, and barleys that are due out over the next few years.

"The initial work against the Russian wheat aphid was landmark research," says the unit's research leader, David Porter. "The best evidence at the time showed that this threat was under control, thanks in large part to ARS research."

Unfortunately, it wasn't. In spring 2003, a new biotype of the pest was found in Colorado and was later identified in several other plains states. This variant was able to overcome many of the resistant wheat and barley lines, as well as all five of the released resistant wheat varieties.

Confronted with seeing their earlier work rendered obsolete, Baker and Mornhinweg turned to an invaluable safety net: the large collection of wheat and barley breeding lines they had developed while fighting the original aphid biotype. They may have hit pay dirt there in the form of advanced germplasm breeding lines also resistant to the new menace.

"If this hadn't been the case, we'd be back at square one," says Baker, whose expertise lies in protecting wheat crops from insect pests. "Hopefully, our discoveries will save us 4 to 6 years of new research."

Readiness Was Deliberate

"This was not by accident," says Mornhinweg, who focuses on protecting barley from pests. "We purposely sought genetic diversity in our resistant breeding lines for just such a possibility."

Mornhinweg says the development may save time in another way. "Usually, once a source of resistance is found, it takes about 10 years to incorporate the resistance into agronomic types appropriate for U.S. farmers," she says.

The discoveries may help thousands of wheat and barley growers envisioning a repeat of the original aphid's damage. That biotype has cost North American producers billions of dollars since its appearance in Texas in 1986.

The Russian wheat aphid is a major pest worldwide of winter wheat, barley, and other cereal crops. This tiny, green bug—about the size of a sesame seed—was first spotted in this hemisphere during the early 1980s, in Mexico.

It feeds on crop plants, causing leaves to curl while producing distinctive white, yellow, and purple longitudinal lines along them. These curled leaves provide shelter for the aphid and make them hard to detect until their damage is apparent. Aphids will still feed on resistant cultivars, but the plants' resistance

PEGGY GREB (K11160-3)

Plant geneticist Cheryl Baker infests greenhouse colonies with the newly found biotype of Russian wheat aphid. These colonies will be used to infest plants that will be screened for resistance to this aphid.

prevents leafrolling; this can significantly lower aphid numbers and reduce plant damage.

Prolific—In Certain Environments

Few farmers were as hard-hit by the pest as those in Colorado. That state's 14,000 wheat growers have suffered through more than $130 million in crop losses and insecticidal control efforts since the original aphid arrived, and its barley industry was brought to a standstill.

PEGGY GREB (K11153-1)

Geneticist Dolores Mornhinweg transfers resistance to adapted U.S. barley by using traditional crossing techniques.

Entomologist John D. Burd of the Stillwater lab says biological, climatological, and ecological factors combine to make Colorado "the economic center of Russian wheat aphid problems in North America.

"This aphid —including the new biotype—reproduces asexually, year-round." Females give birth to live females, and no eggs are involved. "This allows the pests to reproduce quickly and in large numbers. They will give birth every 4 to 6 hours under optimum conditions."

This aphid is very particular about its environment. Conditions cannot be too hot or too cold, and there has to be an abundance of volunteer wheat—from spillage, wind, or harvesting—for oversummering. "The grasslands of eastern Colorado provide all these," says Burd.

The Stillwater lab was among many research institutions that responded to the original Russian wheat aphid threat. In collecting as many wheat and barley germplasm samples as possible to test for aphid resistance, its scientists found their greatest resource to be the vast National Small Grains Collection (NSGC), managed by the ARS Small Grains and Potato Germplasm Research Unit in Aberdeen, Idaho.

NSGC personnel provided accessions for testing and collected and made available test data to barley and wheat researchers worldwide. Overall, scientists from the two labs tested 30,000 wheat accessions and all the available 24,000 barley accessions.

Baker also contacted germplasm collectors overseas—a move that may prove vital in the fight against the new aphid biotype.

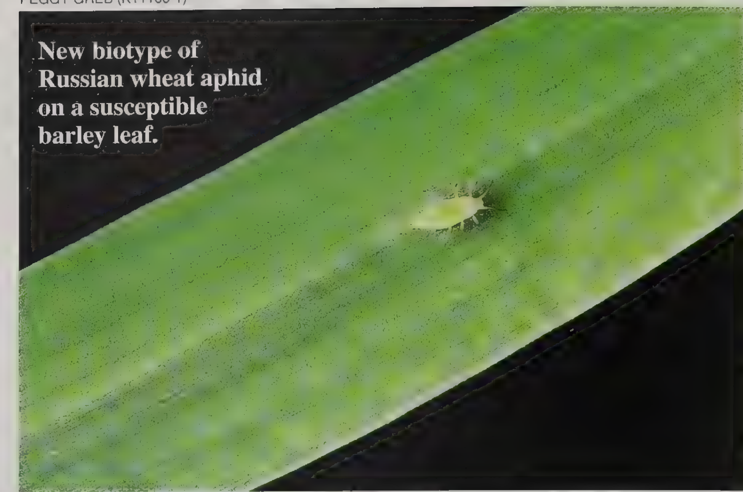
Years of greenhouse testing against millions of aphids led to identification of more than 300 resistant wheat germplasm lines. Mornhinweg developed 40 barley germplasm lines she terms highly resistant to the pest, as well as some lines with intermediate resistance.

The original studies had a huge impact. Baker says the effort has helped many state and private wheat breeders screen their material. "We've also done extensive crossing to incorporate

resistance into wheat lines that have agronomic characteristics suitable for U.S. farmers." She says that virtually all the identified unimproved plant introductions she encountered had characteristics that made them unattractive to American farmers. "They're usually very tall and weak-strawed, and they mature at the wrong time," she says. "But we've bred out the undesirable characteristics and put the aphid resistance into plants with desirable agronomic backgrounds."

Meanwhile, Mornhinweg's work may rejuvenate eastern Colorado's barley industry. She says 62 adapted barley germplasm breeding lines have been developed from her work against the original aphid. ARS geneticists Phil Bregitzer and Don Obert at Aberdeen conducted field testing and selection of advanced breeding lines, and Bregitzer made a few crosses that may be released as germplasm.

PEGGY GREB (K11166-1)



New biotype of Russian wheat aphid on a susceptible barley leaf.

Then, Along Came Trouble

The new aphid biotype threatened much of this work. "The discovery of the new biotype was alarming at first," says Baker. "You can't breed for resistance to a biotype until it arises. But we did our best to prepare for something like this."

The barley germplasm is showing strong promise against the new aphid biotype. "We've tested about one-third of the lines resistant to the original aphid against the new biotype and found all of them to be resistant," says Mornhinweg. She adds that four breeding lines of winter barley and three feed barleys set to be released within the next few years show resistance to both biotypes. These co-releases will occur in conjunction with the Aberdeen lab, University of Idaho, Colorado State University, the University of Nebraska, and New Mexico State University.

Results are still up in the air with most of the wheat germplasm breeding lines. Baker says most sources of resistance to the first biotype have yet to be tested against the new biotype, but so far there are some strong candidates among the advanced breeding lines. Interestingly, the best ones are derived from a wheat-rye translocation line she received from G.F. Marais, a scientist from South Africa, a nation that has problems with the Russian wheat aphid similar to those experienced in Colorado. "It shows a lot of potential," she says. "It looks like aphids don't even want to feed on it."

The Stillwater scientists emphasize that they have received plenty of help. Mornhinweg has been assisted by Bregitzer and Obert, entomologists Frank Peairs and Robert Hammon from Colorado State University, agronomist Dave Baltsenperger from the University of Nebraska, and New Mexico State University associate professor Mick O'Neill.

PEGGY GREB (K11157-1)

Sarah Schultz, biological science aid, transplants winter barley into the greenhouse for seed increase.

Baker has worked with wheat breeding and genetics professors Brett Carver of Oklahoma State University and Stephen Baenzinger of the University of Nebraska, and wheat breeders Kim Kidwell of the University of Washington and Cal Konzak, president and CEO of the Northwest Plant Breeding Company in Pullman, Washington.

Neither ARS scientist could say enough about how much the germplasm collection in Aberdeen helped. "People don't realize how important these collections are," says Baker. "As improved varieties gain popularity and more acreage is planted with fewer varieties, genetic diversity decreases. These gene banks are very important sources of potential genetic diversity, including plants tolerant of drought and other environmental conditions, as well as resistant to insects and disease."—By **Luis Pons,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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Sex Potion Ensnares Mealybugs

Female pink hibiscus mealybug.

The pink hibiscus mealybug (PHM) has a dainty name, but don't let it fool you. This insect pest can destroy more than 200 plant species by injecting them with toxic saliva while sucking their sap. The exotic insect pest recently invaded California and Florida, and has proven difficult to monitor. The mealybugs are also hard to kill, partly because their waxy outer coating, or cuticle, resists penetration by conventional insecticides.

But ARS scientists have now found a way to lure male mealybugs, making them easier to detect. A team of researchers led by chemist Aijun Zhang at the Chemicals Affecting Insect Behavior Laboratory, in Beltsville, Maryland, has discovered two compounds that together make up the female PHM's sex pheromone. The compounds provide a timely method with which to monitor and ultimately reduce infestations.

The scientists carefully reared thousands of PHMs, using an insect growth regulator that prevents development of males. Then they painstakingly isolated more than 6,000 virgin females from which they collected pheromone chemicals.

After pooling seven collections of airborne sex-chemical extracts from the females, the scientists exposed them to male mealybugs' antennae, which consistently responded to two attractant compounds. The previously unknown natural chemicals were found to be (R)-lavandulyl (S)-2-methylbutanoate and (R)-maconelliyl (S)-2-methylbutanoate.

The researchers then prepared a synthetic version of the pheromone and further demonstrated that the processed mixture was immensely attractive to PHM males. They found the most potency when they mixed one part of the first compound with five parts of the second.

"In many cases, just a few micrograms of the one-to-five blend placed in a single sticky trap captured thousands of males," says Zhang. This blend is effective for monitoring the mealybug's population densities and geographical distribution to help scientists determine where to release natural enemies.

"Pheromones decompose relatively quickly, without leaving a harmful residue or damage to the environment," says Zhang. Chemical insecticides, however, break down at a very slow rate, so they tend to linger in soil for decades, which can add to pollution. So biological control methods—where natural predators and parasitoids are "weaponized" against agricultural pests—are more desirable.

Officials with USDA's Animal and Plant Health Inspection Service, headquartered in Riverdale, Maryland, have introduced two exotic wasps to control PHM infestations in the United States and Caribbean. But their efforts had been stalled by an inability to detect the mealybug's presence and prevalence. Now, APHIS officials are using the new pheromone blend as a sex lure to survey the degree of mealybug pest infestations in Florida and California and to track the effectiveness of biological control efforts against the pest.

USDA-ARS has applied for patent protection for the invention and has already received requests to license the technology. The new blend of synthetic pheromones could help crop producers safely manage the pests with either mass-trapping or disruption of mating activity.

These natural compounds have good staying power: Just 1 microgram lures the pest for 6 months, and 10 micrograms lures it for 1 year, Zhang says.

The discovery was published in the *Proceedings of the National Academy of Sciences.*—By **Rosalie Marion Bliss,** ARS.

This research is part of Crop Production, Product Value, and Safety, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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What's Buzzing with

Africanized Honey Bees?

In 1990, a honey bee swarm unlike any before found in the United States was identified just outside the small south Texas town of Hidalgo. With that identification, Africanized honey bees were no longer a problem we would have *some day*. Africanized honey bees had arrived.

Beekeepers, farmers who depend on honey bee pollination for their crops, land managers, emergency responders like fire and police, and the public all wanted to know what they would be facing as Africanized honey bees began to spread.

Now, 14 years later, scientists with the Agricultural Research Service and elsewhere have uncovered many answers, but they have also come upon some new and unexpected questions.

Africanized honey bees—melodramatically labeled "killer bees" by Hollywood hype—are the result of honey bees brought from Africa to Brazil in the 1950s in hopes of breeding a bee better adapted to the South American tropical climate. These honey bees reached the Brazilian wild in 1957 and then spread south and north until they officially reached the United States on October 19, 1990.

Actually, all honey bees are imports to the New World. Those that flourished here before the arrival of Africanized honey bees (AHBs) are considered European honey bees (EHBs), because they were introduced by European colonists in the 1600s and 1700s. EHBs that escaped from domestication are considered feral rather than wild.

Africanized honey bees are so called because it was assumed that the African honey bees spreading out from Brazil would interbreed with existing feral EHBs and create a hybridized, or Africanized, honey bee.

This has always been a major question for researchers—what, if any, type of interbreeding would happen between AHBs and EHBs and how would this affect honey bee traits that are important to people, such as swarming and absconding, manageability for beekeepers, honey production, and temper.

SCOTT BAUER (K11074-1)

Entomologist David Gilley is part of the team investigating the usurpation of European honey bee colonies by swarms of Africanized honey bees. Because queenless colonies are particularly susceptible to usurpation, the team maintains a group of queenless colonies to lure usurpation swarms into their apiary to be studied. Gilley is shown here requeening one of these "bait colonies."

Many experts expected that the farther from a tropical climate AHBs spread, the more they would interbreed with EHBs. But it appears that interbreeding is a transient condition in the United States, according to ARS entomologist Gloria DeGrandi-Hoffman. She is research leader at the Carl Hayden Bee Research Center in Tucson, Arizona, and ARS national coordinator for AHB research.

"Early on, we thought the mixing would reach a steady state of hybridization, because we knew the two groups of bees can easily interbreed and produce young," DeGrandi-Hoffman says. "But while substantial hybridization does occur when AHBs first move into areas with strong resident EHB populations, over time European traits tend to be lost."

A Mighty Adversary

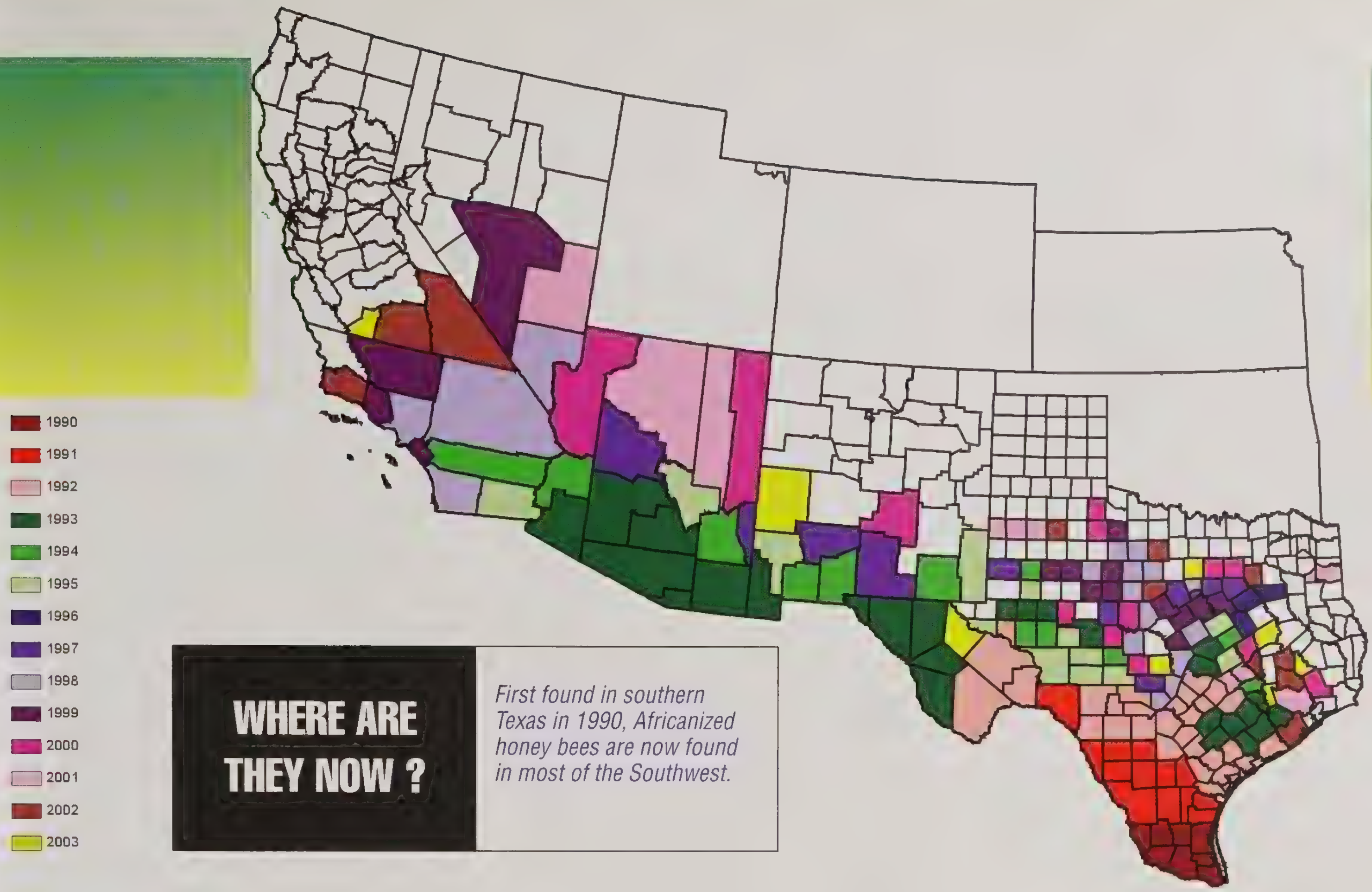
DeGrandi-Hoffman and Stan Schneider, a professor of biology at the University of North Carolina at Charlotte, have been collaborating for 3 years to see why AHBs replace EHBs rather than commingling.

"We've found six biological and behavioral factors we think are responsible for making AHBs such successful invaders," Schneider explains.

First, AHB colonies have faster growth rates, which means more swarms splitting off from a nest and eventually dominating the environment.

Second is that hybrid worker bees have higher amounts of "fluctuating asymmetry"—small, random differences between the left and right wings—than African honey bees have, even when raised in the same hive.

"Imperfections like fluctuating asymmetry that increase with hybridization



may end up reducing worker viability and colony survival," says DeGrandi-Hoffman. "But this is a controversial factor right now, and it will take long-term studies of African, hybrid, and European colonies in the same habitat to truly understand its influence."

But the third factor is undeniably true: EHB queen bees mate disproportionately with African drones, resulting in rapid displacement of EHB genes in a colony. This happens because AHBs produce more drones per colony than EHBs, especially when queens are most likely to be mating, DeGrandi-Hoffman explains.

"We also found that even when you inseminate a queen with a 50-50 mix of African drone semen and EHB semen, the queens preferentially use the African semen first to produce the next generation of workers and drones, sometimes at a ratio as high as 90 to 10," she says. "We don't know why this happens, but it's probably one of the strongest factors in AHBs replacing EHBs."

When an Africanized colony replaces its queen, she can have either African or European paternity. Virgin queens fathered by African drones emerge as much as a day earlier than European-patriline queens. This enables them to destroy rival queens that are still developing. African virgin queens are more successful fighters, too, which gives them a significant advantage if they encounter other virgin queens in the colony. DeGrandi-Hoffman and Schneider also found that workers perform more bouts of vibration-generating body movements on African queens before they emerge and during fighting, which may give the queens some sort of survival advantage.

AHB swarms also practice "nest usurpation," meaning they invade EHB colonies and replace resident queens with the swarm's African queen. Nest usurpation causes loss of European matrilines as well as patrilines. "In Arizona, we've seen usurpation rates as high as 20 to 30 percent," says DeGrandi-Hoffman.

Finally, some African traits are genetically dominant, such as queen behavior, defensiveness, and some aspects of foraging behavior. This doesn't mean that EHB genes disappear, but rather that hybrid bees express more pure African traits. The persistence of some EHB genes is why the invading bees are still considered Africanized rather than African, regardless of trait expression, she points out.

A coincidence may have contributed greatly to an overwhelming takeover by AHBs in areas they've invaded. Just as AHBs began their spread throughout the Southwest, the U.S.

SCOTT BAUER (K11071-1)

An Africanized honey bee (left) and a European honey bee on honeycomb. Despite color differences between these two bees, normally they can't be identified by eye.

feral honey bee population was heavily damaged by another alien invader—the deadly *Varroa* mite, an Asian honey bee parasite first found here in 1987.

"*Varroa* mites emptied the ecological niche of feral honey bees just as AHBs arrived," says DeGrandi-Hoffman. "If they hadn't been moving into a decimated environment, AHBs might not have replaced EHBs so quickly."

Keeping Tabs on the Invaders

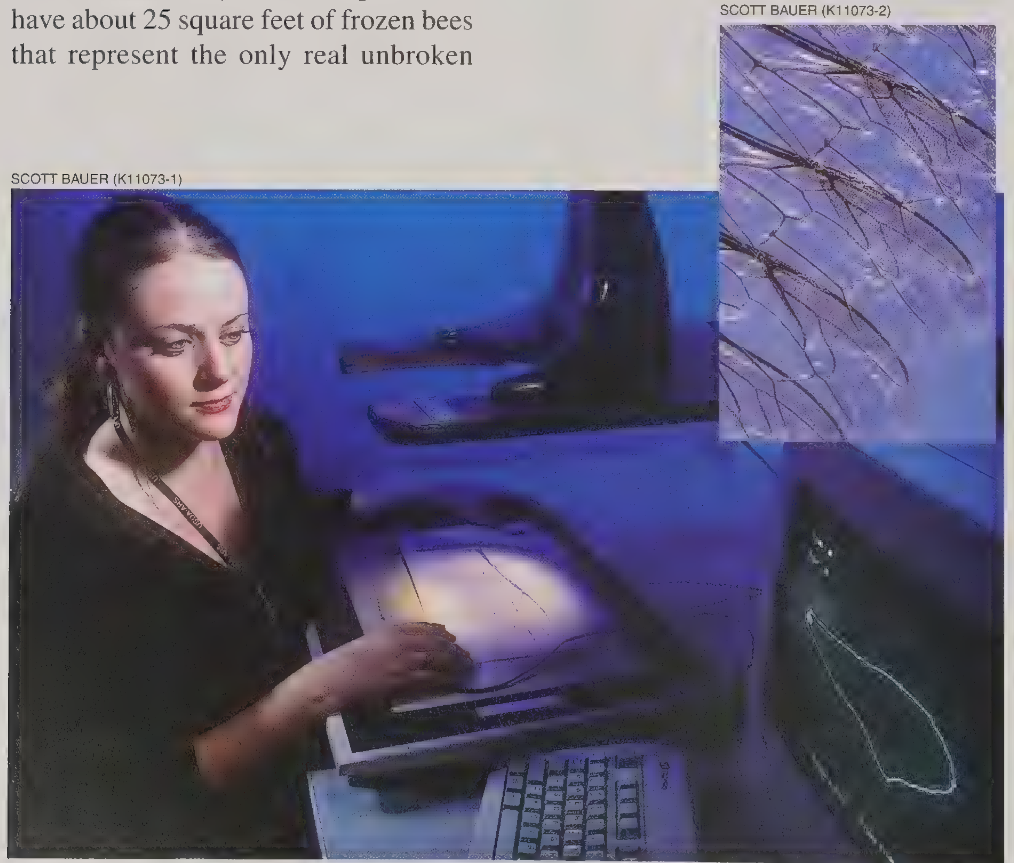
An extensive record of the AHB invasion was created by now-retired ARS entomologist William L. Rubink, who was in the ARS Bee Research Unit in Weslaco, Texas. From 1990 to 2001, Rubink continuously sampled honey bee colonies in the Welder Wildlife Refuge, about 30 miles north of Corpus Christi, Texas.

Once Rubink retired, researchers from Texas A&M University agreed to preserve and analyze his samples. "We have about 25 square feet of frozen bees that represent the only real unbroken sampling of a wild area before and during its takeover by AHBs. Bill had a great deal of foresight to take these samples," explains geneticist J. Spencer Johnston, who is with the university.

The data showed that within 3 years of the arrival of AHBs in the refuge there was a turnover from predominantly EHB to predominantly AHB. From 1997 through 2001, the mixture stabilized, with an average of 69 percent of the colonies made up of African queens mated with EHB and AHB drones and 31 percent composed of EHB queens mated with AHB and EHB drones. This produced a genetic mixture rather than a replacement of EHBs by AHBs. Additional sampling and more analysis of existing samples will be needed to see whether this mixing continues or whether the Africanized proportion increases, as has been predicted.

SCOTT BAUER (K11073-2)

SCOTT BAUER (K11073-1)



Technician Mona Chambers measures the dimensions of an Africanized honey bee wing, one of the traits that differentiate these bees from European bees. Inset: Closeup of the wings Chambers is viewing.

Human Parallels?

In many ways, the spread of AHBs in the Southwest has been one of the most successful introgressions ever documented. It's even interested some as a model of how modern humans may have interacted with the European population of Neanderthals.

"Alan Templeton, a professor of biology and genetics at Washington University in St. Louis, has been looking at AHB spread as a demonstration of his model of *Homo sapiens'* evolution and spread, which holds that there have been three major migrations out of Africa, with large amounts of genetic interchange among groups," Johnston says. Honey bee generations are short enough that you can actually follow the invasion and the gene flow, unlike humans, explains Johnston.

Where Did They Go?

Just how far and how fast AHBs have spread in the United States may be one of the most surprising factors in the whole issue.

Some experts predicted the bees would spread throughout the country; others thought they'd reach only as far north as the latitude of Houston. Most expected there would be a southern zone where AHBs would predominate, a northern zone where EHBs would maintain a climatic advantage, and a large transitional zone between the two. And everyone expected AHBs to spread across the southernmost tier of states. But, as of January 2004, AHBs have been found only in southern California, Arizona, New Mexico, Nevada, and Texas, as well as Puerto Rico and the U.S. Virgin Islands.

Why AHBs haven't progressed eastward into Louisiana—though they were expected there years ago—is a mystery. So ARS entomologist José D. Villa began looking at factors that might correlate with where AHBs have spread. It isn't just minimum winter temperature that limits AHB spread, as many believed,

says Villa, who is in the ARS Honey Bee Breeding, Genetics, and Physiology Research Unit in Baton Rouge, Louisiana.

"What immediately jumped out at me was the correlation with rainfall," he says. "Rainfall over 55 inches, distributed evenly throughout the year, is almost a complete barrier to AHB spread."

Total annual rainfall alone isn't a barrier; AHBs have been found in areas of the Tropics with higher rainfall. But in areas with high rainfall distributed throughout the year, Villa's pattern of AHB spread fits perfectly.

Villa is quick to point out that this is simply a mathematical correlation and not proof of cause and effect. But, he says, "you do find that 55-inches-of-rainfall point right at the edge of where AHBs stopped moving east about 10 years ago." He's planning experiments that may uncover the behavioral or physiological mechanism that explains why.

How much farther AHBs may spread is still unknown. But if you apply the 55-inches-of-rainfall limit, there are still niches that the bees may fill, mainly in southern California. Southern Florida would be hospitable to the bees given its temperature and rainfall, but regulatory vigilance could keep them out, since the area isn't contiguous with the other areas of AHB spread. Alabama, northern Florida, Louisiana, and Mississippi are unlikely to be troubled by AHBs if the 55-inches-of-rainfall barrier holds.

Keeping on Beekeeping

One of the greatest challenges for Southwest beekeepers has been maintaining their EHB hives when they are surrounded by AHBs.

Once AHBs spread to an area, beekeepers can no longer allow nature to take its course in honey bee reproduction. ARS has always recommended that beekeepers regularly requeen their hives with queens of known lineage to keep AHB traits out of their apiaries. But, given the African bees' strong ability to genetically usurp hives, the recommendation is now to requeen with queens that have already mated with EHB drones. It's the best way ARS currently has for beekeepers to manage their hives in AHB areas.

But requeening is a lot of work for commercial beekeepers who maintain thousands of hives. DeGrandi-Hoffman and Schnieder are currently trying to discover what triggers AHBs to usurp a hive. They suspect it could be a pheromone.

"If we can find out what tells an AHB swarm that this EHB nest can be taken over or that a colony or queen is strong and cannot be easily usurped, then we should be able to develop a chemical 'no-vacancy' sign to help beekeepers keep AHBs out," DeGrandi-Hoffman says.

While AHBs do make honey and pollinate plants, two traits make them undesirable for beekeepers: Colonies regularly abscond from hives, and they are often too defensive to be easily tended.

Because of AHBs' genetic dominance there has been little dilution of their strong defensive reaction to threats to their nests, explains DeGrandi-Hoffman. This defensiveness is probably the bees' best-known trait. All honey bee behavior runs the gamut from very defensive to very docile and can change depending on temperature, humidity, cloud cover, and food supply. But when provoked, AHBs do tend to sting in greater numbers than EHBs.

"But they're not anywhere near the type of threat that Hollywood has made them out to be," DeGrandi-Hoffman points out.

Living with AHBs

While beekeepers obviously do not want to work with "hot bees," people in the Southwest have simply learned to live with AHBs. While many will never come in contact with the bees, others have had to learn new precautions.

Retired ARS entomologist Eric Erickson, who was with the ARS bee center in Tucson, pioneered many safety methods in areas where people and AHBs collide. He developed the first instructions for fire departments—often the emergency

JACK DYKINGA (K7937-10)

Northwest Fire District's Captain John Estes of Tucson, Arizona, uses a wide spray of water and chemical wetting agent as a means of subduing Africanized honey bees. Looking on is ARS entomologist Eric Erickson (retired), who taught this control method to fire departments throughout Arizona.

responders in stinging incidents. Most firetrucks already carried a surfactant, a soapy liquid that helps put fires out. Such soaps also kill honey bees when sprayed directly on them. Erickson also worked out ways to quickly convert a firefighter's basic turnout gear into a protective bee suit. Fire departments all over the Southwest are now trained in Erickson's methods.

Erickson also developed instructions for homeowners to help them deal with AHBs, such as how to prevent honey bees from taking up residence inside house walls and how to kill unwanted bee colonies. (It is safer, though, to call an experienced exterminator if at all possible.)

Swarm traps invented by entomologist Justin O. Schmidt, also at the Tucson bee center, have been a boon.

"We developed a simple, inexpensive trap with a pheromone lure to attract swarms looking for new nest sites. That's how we're able to track honey bee colonies as they spread out," Schmidt says.

The traps are also used as prophylactic barriers around golf courses, airports, schools, and botanic gardens, or anywhere else AHBs might take up residence and conflict with people. The traps lure swarms away from high-traffic areas and make them easy to remove.

Not All Bad

People usually think only of AHBs' downside, but they also represent a potential positive. ARS entomologist Frank A. Eischen at the Honey Bee Research Unit in Weslaco, Texas, has been studying AHBs for their resistance to *Varroa* mites.

Eischen maintains an apiary in a remote part of southern Texas. "Maintains" may not be the right term, because he simply leaves hive boxes out and lets the bees fend for themselves year after year. All the honey bees in the apiary have long since been Africanized.

His AHBs, which are never treated, have a slightly better survival rate against *Varroa* mites. But that rate varies dramatically.

"I've looked at about 40 colonies. Some have very few mites, and others are loaded," Eischen says. "But if these had been EHB colonies without treatment, they all would have died long ago."

He is trying to isolate which mechanism provides the protection from *Varroa* mites. He has already ruled out hygienic behavior—the time it takes worker bees to clean out mites. But if he determines what AHBs do differently, it might be possible to breed that desirable trait into EHBs.—By **J. Kim Kaplan,** ARS.

This research is part of Crop Production, an ARS National Program (#305) described on the World Wide Web at www.nps.ars.usda.gov.

To reach scientists mentioned in this story, contact Kim Kaplan, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5128; phone (301) 504-1637, fax (301) 504-1648, e-mail kaplan@ars.usda.gov. ★

SCOTT BAUER (K11076-1)

Entomologist Justin Schmidt examines an ARS honey bee trap used to lure Africanized bee swarms and prevent their establishment in walls of buildings. Captured swarms are easily removed or destroyed with soapy water.

SCOTT BAUER (K11075-1)

Judith Hooper assists David Gilley with a gas chromatography/mass spectroscopy procedure for analyzing volatile pheromones from Africanized honey bees. Pheromones appear to be an important component of successful nest usurpation.

Greenhouse Pests Beware

Old World Hunter Fly Now in North America

There's bad news for many insects that pester North American greenhouses. Scientists from Cornell University and ARS have reported the first-ever identification on the continent of the Old World hunter fly, *Coenosia attenuata*.

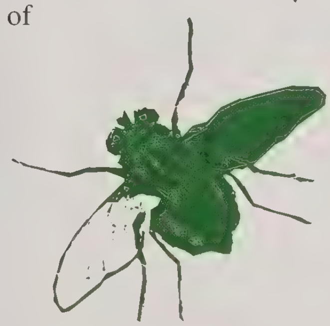
This winged predator from Europe, where it's also known as the "killer fly," has a taste for some of the insects greenhouse keepers find most distasteful: fungus gnats, shore flies, leafminers, fruit flies, moth flies, and some leafhoppers.

"Hunter flies were originally described in southern Europe," says ecologist Steve Wraight. He's in ARS's Plant Protection Research Unit, which is based on Cornell's Ithaca, New York, campus. "But they were found in South America before they were seen here. So, this fly's apparently been on the move for quite a while."

In fact, hunter flies have been reported in southern Asia, northern and southern Africa, the Canary Islands, New Guinea, and Australia, as well as in the Americas. "We're not certain exactly how it got to the New World," says Wraight, "but it may have come over via the horticulture industry, in soil or plant material."

Hunter fly research is focused on increasing our knowledge of fly biology, including predatory behavior and rearing requirements. It is funded through USDA's Floriculture and Nursery Research Initiative. Initial studies were conducted by Cornell graduate student Emily Sensenbach, under the direction of Wraight and Cornell associate professor John Sanderson. *C. attenuata* is from the same insect family as the common house fly (Muscidae) and is similar in appearance. But this fly lives up to its name—and not just because it preys on other flying insects.

The Old World hunter fly seemingly enjoys a challenge. "They sit, wait, and only pursue prey that are in flight," says Wraight. "If an insect is not flying, they won't chase it, even if it's close by." The flies catch their targets, puncture them with a daggerlike mouthpart, and consume their innards. They can turn cannibalistic when the pickings get scarce. The hunter fly's soil-dwelling larvae are also predatory, feeding mainly on larvae of other insects, including fungus gnats.



Hunter flies colonize greenhouses by settling into greenhouse soil. The insect was first noticed in Onondaga County, New York, in 1999 by Elise Schillo-Lobdell, a contractor working as a greenhouse scout, at a commercial greenhouse specializing in large-scale poinsettia production. Specimens were sent to Cornell in 2002 and identified by senior extension associate Richard Hoebeke. Since then, many more specimens have been collected on sticky-card traps set for monitoring pest populations in other greenhouses across New York, as well as at a site in Ontario, Canada. They've also been collected from a trap in Los Angeles County, California.

Wraight sees considerable potential for using hunter flies in biological control. "We could start by developing methods to increase populations of flies already in the greenhouse," he says. "This might be done by providing a stable soil environment to ensure survival of the fly's immature stages."—By **Luis Pons,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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Scientists Target Glassy-Winged Sharpshooter

Just as your home has pipes that move water into it, plants, too, have their own plumbing systems. In plant tissue called xylem, water and nutrients flow from roots to stems, branches, leaves, buds, blooms, and fruit.

But just as household water pipes can clog, so can plants' plumbing. Severe clogs can cause plants to weaken and die.

A half-inch-long leafhopping insect called the glassy-winged sharpshooter can inadvertently plug plants' plumbing. When it shoves its tubelike mouthparts into a plant to suck sap from the xylem, the insect may transmit a deadly plant bacterium, *Xylella fastidiosa,* in its saliva.

This microbe can live in the sharpshooter's gut without harming the insect. But when *Xylella* moves from the sharpshooter into a plant, the bacterium can form colonies or clusters that may eventually shut off the flow of water.

The condition that *X. fastidiosa* causes in grapevines is known as Pierce's disease. Southern California winegrape vineyards got hammered with it in the 1990s soon after this sharpshooter first appeared in the Golden State.

Agricultural Research Service scientists across the United States teamed up to devise environmentally friendly, science-based strategies to help check the spread of the pesky insect and the devastating disease. *(See box, facing page.)*

That work paved the way for new studies that may unlock secrets about the complicated interaction between the insect, bacterium, and vines. An example: the innovative investigations conducted by a sharpshooter squad of scientists with the ARS Exotic and Invasive Diseases and Pests Research Unit, Parlier, California.

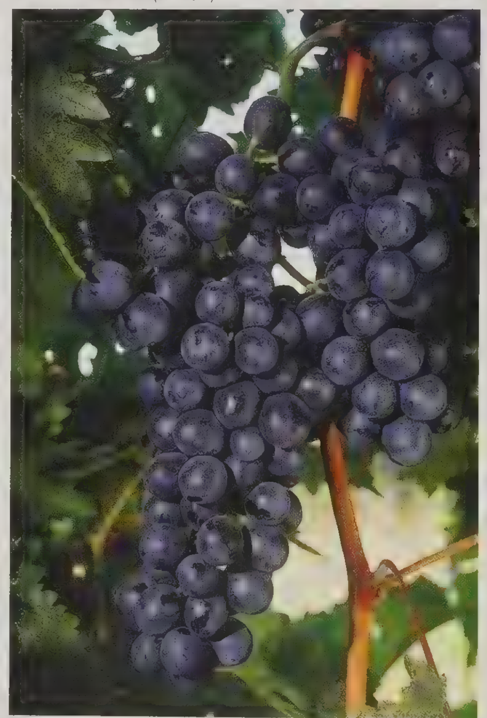
"Wired" Takes on a New Meaning

A thin gold wire attached to a sharpshooter's back may enable scientists to zero in on the minute-by-minute actions of the pest as it attacks plants. "The wire carries a low-level electric current," explains ARS entomologist Elaine A. Backus. The plant is also wired, so the circuit is completed when the insect punctures the grapevine to get a drink.

A pattern of electrical waves, somewhat like an electrocardiogram, is recorded as the thirsty insect sips its fill. From these charts, Backus intends to piece together new clues about exactly when, how, and how quickly the *Xylella* microbes in the insect's gut get dislodged and shuttled into the vine.

The work may also shed light on variations in grapevines' natural resistance to the invasive insect and the bacterium. The work may also become the basis for a quick, reliable way to screen vines for superior resistance.

PATRICK TREGENZA (K5633-7)



Grape genes for resistance to Pierce's disease are the focus of some ARS studies in Parlier, California.

Studying Sharpshooter Biology and Ecology

No one can say for certain where sharpshooters are most likely—at any given time of the year—to rest, feed, lay their eggs, or, perhaps most importantly, pick up *Xylella*.

To fill this gap, entomologist Russell Groves is creating a detailed picture of what he describes as "seasonal dispersal of sharpshooters among various kinds of vegetation in the landscape." He has installed an extensive network of traps that he monitors once a week, year-round. The traps consist of bright-yellow cardboard coated with a sticky compound.

"Sharpshooters are attracted to the colored panel," says Groves. "When they fly close to investigate, they can't pull free of the sticky coating."

Groves' traps run in lines from streambanks to weedy fencerows to plants in fields, orchards, and vineyards. "Growers will get the most from their pest-control dollars," says Groves, "if they know what plants are *Xylella* reservoirs and what plants sharpshooters will target next."

Genes May Proffer Protection Against Pierce's Disease

Thanks to their genes, some plants are better able than others to shrug off attack by the sharpshooter and *Xylella*. Genes may cue plants to make natural compounds that repel the insect or blunt *Xylella*'s ability to infect.

Plant physiologist Hong Lin and University of California at Davis co-investigators are hunting for these genes. At a research greenhouse in Davis, they've inoculated hundreds of grapevines with *X. fastidiosa*. "The plants we're using," says Lin, "are already known to be either resistant or susceptible to *X. fastidiosa* infection."

The scientists remove tissue from the grapevines at regular intervals. The sampling spans only 4 to 5 months because by then vulnerable vines have been killed by Pierce's disease. "We're looking at the types and amounts of gene products—such as proteins—these plants form," explains Lin. "We want to see if there's any significant antimicrobial effect in these compounds. That could lead us to the genes that direct the plants to make the compounds."

REYES GARCIA III (K10693-1)

The glassy-winged sharpshooter is the culprit behind the spread of Pierce's disease among grapevines. The insect infects the plant with the bacterium *Xylella fastidiosa* when it feeds on the sap from the xylem tissue of a vine.

It might then be possible to move those genes into commercial rootstocks, he notes. A rootstock is the bottom, rooted portion of the plant to which the upper, grape-bearing scionwood is grafted.

The search for resistance genes is greatly helped by the availability of worldwide databases that depict the genes responsible for disease resistance in other green plants, says Lin. Using what others have already learned and made available about disease-resistance genes accelerates discovery of similar genes in plants such as grapevines.

The Many Faces of *Xylella*

It's not just grapevines that are beleaguered by *X. fastidiosa*. The microbe occurs in many forms, or strains, that sicken other plants, including almond, peach, plum, and oleander.

So what's the best way to sort out who's who in the world of *Xylella*?

Why not examine their genetic material, or DNA, advises Jianchi Chen, ARS molecular biologist. A test based on *Xylella* genetic material could be ideal for determining which *Xylella*—if any—newly invading sharpshooters are carrying. That "inside" information could give growers a heads up.

Too, such a test could be used to screen imported grapevines to be sure they're free of *Xylella*. Right now, plants sometimes have to be monitored for weeks or months to be certain they're disease free.

The Parlier research weaves together different kinds of scientific expertise to unravel the destructive interactions of an insect, a pathogen, and a vulnerable plant. This research and that at a half-dozen other ARS locations around the nation (see *Forum*, page 2) should yield new, effective tactics to minimize the menace posed by *Xylella* and the sharpshooters.—By **Marcia Wood,** ARS.

This research is part of Crop Protection and Quarantine (#304) and Plant Diseases (#306), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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Fast-Thinking First-Responders Garner USDA Honor Award

From coast to coast, ARS scientists have pooled their expertise to slow the spread of the glassy-winged sharpshooter and the disease-causing *Xylella fastidiosa* microbes it carries.

The researchers' aggressive, timely responses to the agricultural emergency caused by the invasion of this insect pest into southern California vineyards earned the scientists and their federal, state, and corporate teammates a USDA Honor Award in June 2003.

Honoree Kevin J. Hackett, an ARS National Program Leader in Beltsville, Maryland, helped orchestrate the work of the ARS specialists on the emergency response team.

Edwin L. Civerolo, now director of the ARS San Joaquin Valley Agricultural Sciences Center, Parlier, California, spearheaded a novel collaboration with Brazilian experts to detect the genetic material of the pathogenic *X. fastidiosa* microbe that the sharpshooters transmit. West Virginia-based entomologist Gary J. Puterka codeveloped kaolin-clay-derived repellant, giving growers an environmentally safe compound to discourage sharpshooters from chewing, sucking, or laying their eggs on vulnerable vines.

Entomologists Thomas J. Henneberry and David H. Akey at Phoenix, Arizona, conducted extensive studies that identified the most effective insecticides for killing sharpshooters, including ecologically sound pyrethroids and neonicotinoids familiar to home gardeners.

Studies organized by Walker A. Jones, an entomologist based at Weslaco, Texas, spotlighted beneficial, stingless wasps with impressive potential to clobber the sharpshooter. *(See story, page 18.)*

New studies in progress in these laboratories and others around the country may reveal yet more tactics to trounce the troublesome insect and the *Xylella* disease it spreads.—By **Marcia Wood,** ARS.

ARS Leads in Assessing Risk in Transgenics

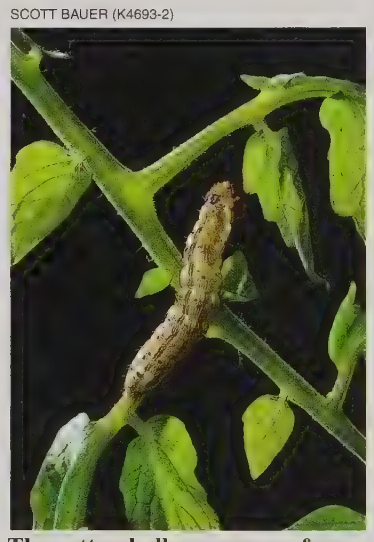
Since before Mary Shelley published "Frankenstein" in 1818, people have oscillated between concern that what scientists create in the lab will be dangerous and hope that research progress will improve their lives.

But few scientific advances have created a wider spectrum of public debate than genetic engineering of living organisms. Many people see the importance of the technology and believe it is essential for developing new and improved agricultural products. Others object to genetic engineering on philosophical grounds or worry about the risks a genetically engineered organism (GEO) could present to people or the environment.

Some people feel that scientists have not paid enough attention to potential risks. If GEOs are to maintain and increase their acceptance as new traits are introduced into more and more species, risk must continue to be clearly and openly assessed.

The assessment of safety data is integral to the regulatory process of the three primary federal agencies responsible for regulating GEOs: USDA's Animal and Plant Health Inspection Service, the U.S. Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA). Advances in our methods of carrying out genetic engineering and in our understanding of physiological and ecological processes allow scientists to maintain sophisticated and state-of-the-art procedures and controls for ensuring the safety of GEOs before they're allowed to be commercially raised.

SCOTT BAUER (K4693-2)



The cotton bollworm, one of several major cotton pests controlled by *Bt* cotton.

There's no question that GEOs are becoming essential to agriculture by making new traits available, helping agriculture be more environmentally sensitive, and reducing production costs. To remain competitive and environmentally sensitive, farmers need traits such as the insect and herbicide resistance offered only by transgenic crops.

For all these reasons, ARS has become a leader in biotechnology risk assessment research.

"For the past 4 or 5 years, ARS has coordinated and carried out more and more biotechnology risk assessment research and directed more resources into this work," says John W. Radin, ARS national program leader for plant physiology and risk assessment. "We've always done some research in this area, but today it's a very high priority."

There are several areas of risk assessment that ARS is uniquely suited to study: creating more specific ways to transfer only desired genes, developing new models for doing risk assessments, finding ways to limit spread of transgenes, discovering ways to prevent new allergens from being created, ensuring that nontarget organisms are not put at risk by a GEO, and carrying out long-term monitoring to spot any emerging resistance to transgenic traits.

Making Sure Resistance Is Futile

Cotton was one of the first crops to benefit from laboratory genetic engineering. Genes from the bacterium *Bacillus thuringiensis (Bt)* were added to cotton, making the plant produce a protein toxic to several major cotton pests, including pink bollworm, tobacco budworm, and bollworm. Control of such pests had previously necessitated massive amounts of pesticide use.

Since EPA approved its release in 1995, *Bt* cotton has been extremely successful in the United States and other countries such as China, India, and Australia. In 2001, transgenic varieties generated an additional $235.6 million in revenue for farmers while reducing pesticide use by 8 million pounds, according to a study by the National Center for Food and Agricultural Policy.

But there's concern that widespread growing of *Bt* cotton may lead to insects developing resistance to *Bt* proteins, thereby canceling out one of the most potent but more environmentally friendly antipest tools. Resistance to foliar-applied *Bt* has shown up in Indianmeal moths, diamondback moths, and at least nine other insects.

So, even though there's been no indication of resistance being generated by *Bt* cotton, EPA requested that monitoring studies be done. Each year, samples of insects are collected in fields all over the Cotton Belt and sent to the ARS Southern Insect Management Research Unit in Stoneville, Mississippi.

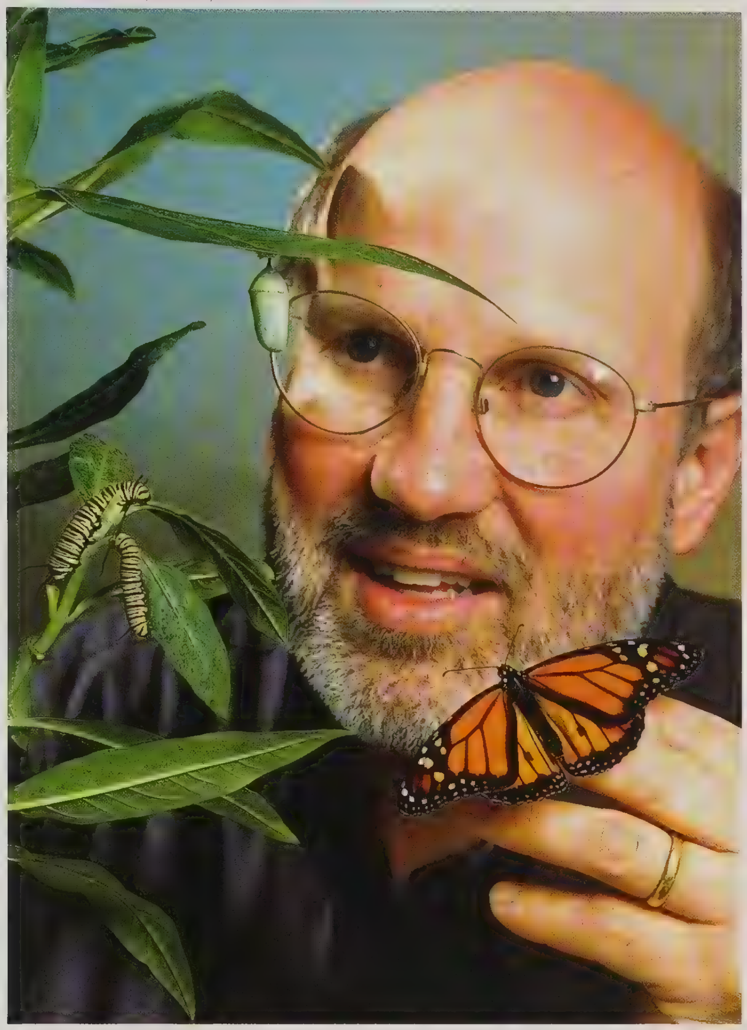
"ARS is the perfect agency for conducting such a long-term, widespread monitoring study that will pick up the first signs of any insect resistance," says John Adamczyk, who, along with Carlos Blanco, coordinates the effort. Both are ARS entomologists.

"ARS is national in scope, which helps when you are running a program that needs to extend from Virginia to Texas," Adamczyk explains. "We're even working on making this an international program, since the insects migrate from Mexico as well."

But perhaps most importantly, he adds, ARS is an unbiased source of data. The agency has no financial stake involved if transgenic cotton is found to be creating a risk of insect resistance.

"We report our results every year, and if we ever do start finding resistance, the industry is reassured that we have no agenda to simply take a technology away," says Adamczyk. While the impetus for resistance monitoring came from

STEPHEN AUSMUS (K11387-1)



Entomologist Richard Hellmich, in the ARS Corn Insects Research Unit in Ames, Iowa, conducted research to confirm what risk, if any, *Bt* corn was to a nontarget insect like monarch butterflies.

STEPHEN AUSMUS (K11332-5)

Technician Michelle Mullen and entomologist John Adamczyk collect bollworm caterpillars from a field of *Bt* cotton at the ARS Southern Insect Management Research laboratory in Stoneville, Mississippi, to test for *Bt* resistance.

industry's need to provide EPA with data, Adamczyk sees the program as serving a wider audience. "We're providing a service to a $10-billion-a-year agricultural industry, but we are really protecting the public and the environment."

The group is also developing better methods that may serve as models for resistance monitoring in conventional pest controls as well as in transgenic crops.

"We're also working on identifying genes that may control insect resistance to *Bt,*" Adamczyk says. "If we can develop better information about that, we may be able to predict resistance very early—before we lose the effectiveness of *Bt*. Such a warning may allow us to do something about it in time."

No Risk to Monarchs

ARS's ability to be the objective voice, not beholden to any one group's agenda, allows the agency to work well with everybody. When a letter published in the May 1999 issue of *Nature* suggested that *Bt* corn threatened monarch butterflies, ARS was able to quickly coordinate groups with widely differing positions on GEOs to develop verifiable, sound, scientific data before any decisions were made, despite an initial flurry of media coverage and public concern.

The concern was that monarch caterpillars eat only milkweed leaves, which sometimes grow in and around cornfields, and that *Bt* corn pollen falls on the milkweed leaves a short time each year.

"Groups from the Union of Concerned Scientists to the Biotechnology Industry Organization, from universities to Monarch Watch, were willing to work with ARS to ensure we really did find out what risk, if any, *Bt* corn was to a nontarget insect like the monarch butterfly," says ARS entomologist Richard L. Hellmich. He's in the Corn Insects and Crop Genetics Research Unit in Ames, Iowa.

How the issue was handled is being seen as a model for nontarget risk assessment research. First, the primary questions were researched. One: What dose of *Bt* protein from the transgenic corn varieties is actually toxic to monarch caterpillars? Two—and just as important: What are the chances that the caterpillars will actually be exposed to that dose?

The science showed that while a toxic dose is reachable, the potential for exposure is insignificant.

"The final consideration," Hellmich says, "is to compare the potential for risk from using the GEO to the alternative—in this case, growing conventional varieties and spraying them with insecticides. Certainly, chemical insecticides kill many more nontargets like monarchs than *Bt* corn does."

Not Spreading the Genes

Another concern widely discussed is ensuring that certain types of transgenic plants do not spread their new genes throughout the environment.

Plant molecular geneticist David Ow, with the ARS Plant Gene Expression Center in Albany, California, is exploring ways to manipulate the DNA of genetically altered plants so that the transgene is deleted or inactivated during the physiological process of pollen production.

"After all, it's not really the presence of the gene itself that's the concern, it's what the gene will do if it spreads to unintended hosts," he explains.

If Ow can work out an effective technique, it could help decrease the potential for risk in all transgenic plants. "That's one of the reasons for ARS to do this kind of work. As a federal agency, we can allow anyone developing a transgenic plant to use the technique, because the public benefits when we decrease risk," he says.

JACK DYKINGA (K5141-3)

Scientists at the ARS Plant Gene Expression Center in Albany, California, were the first in the world to genetically engineer barley.

Another ARS plant molecular geneticist, James E. Dombrowski, with the Forage Seed and Cereal Research Unit in Corvallis, Oregon, is approaching the problem from a different angle. He wants to find a way to inhibit flowering in grass and forage crops. In addition to preserving much of a plant's nutritive value, no flowering would also mean no pollen and no seeds, which would virtually eliminate the chance of transgene spread. He has already identified some flowering genes in grasses.

Dombrowski believes genetic engineering has great potential benefit, but he strongly advocates including risk assessment in transgenic research, "especially with plants like grasses that are wind pollinated and have the potential to cross with other plants," he says.

"We strive to have solid information about what happens with transgenic organisms in the real-world environment, not just in the lab or under controlled conditions. We need solid facts, like how far pollen drifts, its fertility lifespan, and its competition level with other pollen. Some of the data must be collected out in the fields under production conditions to give the real picture of potential risk."

Dombrowski says the public has a legitimate right to expect scientists to be concerned about the potential risks of transgenic crops. But, he adds, "I believe there's a lot of unwarranted fear due to a lack of communication. And in some cases, people aren't really thinking the issues and arguments fully through.

"For instance, you take a gene from rye and put it into wheat to give it resistance to a rust disease, and people are suddenly concerned about what they're eating. But people eat seven-grain bread with wheat and rye in it every day. And in doing so, they're already consuming the combined DNA and proteins from both plants."

New Genes, New Allergies?

Concerns about creation of new allergens are legitimate, and checking this out has always been part of the regulatory approval

Genetic Engineering Timeline

You can date the history of genetic engineering several ways, considering how long people have been manipulating genes.

STEPHEN AUSMUS (K11431-1)

8000 B.C. Humans domesticate crops and livestock and begin selecting for superior traits.

1863 A.D Gregor Mendel discovers traits are inherited through discrete, independent units (genes) and in specific, predictable patterns.

1906 Term "genetics" is introduced.

1919 First use of the word "biotechnology" in print.

DOUG WILSON (K7188-18)

1933 Hybrid corn is commercialized, eliminating the option of saving seeds. Remarkable yields outweigh increased costs of annual seed purchases, and by 1945, hybrid corn accounts for about 78 percent of U.S.-grown corn.

1941 Term "genetic engineering" is first used by Danish microbiologist A. Jost in a lecture on reproduction in yeast in Lwow, Poland.

1946 Discovery that genetic material from different viruses can be combined to form a new type of virus, an example of genetic recombination.

process. The assessment of the potential for new allergens in food is integral to the FDA process for reviewing transgenic plants.

"The public has the right to feel confident about its protection," says ARS molecular biologist Eliot M. Herman at the Donald Danforth Plant Science Center in St. Louis, Missouri. "As we learn ever more about biological systems, we can provide even more specific assurances. Risk assessment will always be an evolving process."

On the other hand, genetic engineering can actually make a food less allergenic. Herman did so when he created a hypoallergenic soybean variety that should not affect the 6 to 8 percent of children and 1 to 2 percent of adults who are allergic to soy. He used a technique called "gene silencing" to shut down the gene that codes for the protein thought to cause most soybean allergies in humans.

SCOTT BAUER (K4389-1)

Using gene silencing, ARS scientists have shut down the genes in soybeans that make the protein thought to cause most soybean allergies in humans.

So far, Herman has tested his hypoallergenic soybean with human sera and in sensitive animals. Testing to be sure allergens are not present is a difficult task. He is currently working with the University of Arkansas Medical School to develop an animal model that will allow for very sensitive allergen testing at the biochemical and cellular level. Such a model would be more explicit and a good addition to the feeding trials now required.

One of the newest areas of genetic engineering is seeking to add to the nutritional value of crops. Herman is looking for new genetic, genomic, and proteomic methods to improve protein, oil, and nutritive value in soybeans.

"While we focus on modifying crops to enhance their nutrition, we also look at genetic expression on a global physiological basis to detect any unpredicted negative effects," explains ARS plant physiologist Leon V. Kochian, with the U.S. Plant, Soil and Nutrition Laboratory in Ithaca, New York.

He points out that if genetic engineering does have negative effects, they are most likely to be seen first in yield losses. "That would direct us to look further at changes," he adds.

Kochian believes strongly in today's increased risk assessment. "Ten years ago, risk assessment research was largely a responsibility of the private sector. But increasingly, public research organizations like ARS have been getting involved. Two important reasons are, one, that USDA research can provide direct support for the needs of the regulatory agencies and, two, that many crops now being genetically engineered are small-market crops, such as fresh fruits. The reasons for making these crops pest resistant and reducing pesticide use are compelling, but companies are reluctant to pursue them because the small amount of acreage involved in growing these small-market crops may preclude profitability."

1973	Stanley Cohen and Herbert Boyer perfect techniques to cut and paste DNA (using restriction enzymes and ligases) and reproduce the new DNA in bacteria.
1980	U.S. Supreme Court rules that genetically altered life forms can be patented and allows Exxon to patent an oil-eating microorganism.
1981	ARS develops foot-and-mouth disease vaccine—the first effective subunit vaccine for any animal or human disease using gene splicing.
1982	Genentech, Inc., receives approval from FDA to market genetically engineered human insulin.
1984	First transgenic farm animals—sheep and pigs—are born.
1986	EPA approves release of the first genetically engineered crop, gene-altered tobacco.
1987	ARS develops microinjection technique to move a whole chromosome into a single cell of another plant.
1991	ARS develops gene-deletion technology to remove antibiotic resistance genes.
1994	FDA approves Flavr Savr tomato, the first genetically engineered food.
1995	*Bt* cotton and *Bt* corn get first EPA approval; registration was renewed in 2001.

BOB BJORK (K2635-9)

Not Just Plants

Plants, of course, are not the only life forms that have been genetically engineered. Livestock, insects, and microorganisms are being genetically tailored for traits that cannot otherwise be easily bred in.

ARS animal physiologist Robert J. Wall with the Biotechnology and Germplasm Laboratory in Beltsville, Maryland, led the collaborative team that, in 2000, succeeded in adding genes for mastitis resistance to a cloned Jersey cow. He served as a subject matter specialist in the USDA Biotechnology Risk Assessment Grants Program workshop on research needs and priorities for animals last year.

"A major difference in risk assessment for genetically engineered farm animals is that we don't have the same worries about transgenes escaping from them as we do with plants," Wall explains. "But we still need to make sure the meat and milk are safe to eat."

The type of risk assessment needed is really determined by the kind of genes that have been added. "If what you add to a Hereford is an extra copy of a bovine growth hormone gene so that muscling is increased, that'll need a lot less testing than adding bacteria genes that don't exist in the cow naturally," Wall says.

"And if the genes are for a product that's broken down in people's stomachs, that too will change the nature of the risk assessment. But the public is entitled to know that we have considered the risks in whatever we are engineering."

That's the key to the future of genetically engineered organisms: The public must know that researchers have competently assessed any risk and that safety has been ensured.—By **J. Kim Kaplan,** ARS.

This research is part of Plant Biological and Molecular Processes, an ARS National Program (#302) described on the World Wide Web at www.nps.ars.usda.gov.

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SCOTT BAUER (K9348-1)

Born March 3, 2000, Annie is the first of what researchers hope will be a new breed of dairy cow whose udders have a bioengineered defense against mastitis disease.

SCOTT BAUER (K9975-3)

Professor Rick Helm (right) and technician Gael Cockrell (center), both with the University of Arkansas, perform an allergy test on the skin of an anesthetized soybean-sensitive pig as ARS plant molecular biologist Eliot Herman observes. The allergy test is similar to that used with humans.

Learning from our elders

Folk Remedy Yields Mosquito-Thwarting Compound

Regional wisdom once imparted by a Mississippi grandfather has led ARS scientists to isolate a natural compound that in laboratory tests was effective in warding off mosquito bites.

The efficacy of the isolated compound—called "callicarpenal"—was affirmed through tests simulating human skin. But these results may not have been a surprise in northeastern Mississippi as long as a century ago, once the source of the callicarpenal was revealed.

Seems that it was known there that fresh, crushed leaves of American beautyberry, *Callicarpa americana,* in the family Verbenaceae, helped keep biting insects away from animals such as horses and mules. Placing crushed beautyberry leaves under the animals' harnesses, residents knew, would mash out a repellent oil. Eventually, some folks there took to mashing the leaves and rubbing the residue on their own skins.

Privy to this knowledge was young Charles T. Bryson, who was told about it by his granddad, John Rives Crumpton.

Today, Bryson is a botanist in ARS's Southern Weed Science Research Unit at Stoneville, Mississippi. And he's told researchers in ARS's Natural Products Utilization Unit at Oxford, Mississippi, about beautyberry's powers.

This led Oxford chemist Charles Cantrell—with entomologist Jerome Klun of ARS's Chemicals Affecting Insect Behavior Research Laboratory in Beltsville, Maryland, and Oxford plant physiologist Stephen Duke—to isolate from American beautyberry and a Japanese counterpart, *C. japonica,* five insect-repelling compounds.

Among them was callicarpenal, which may represent ARS's next important contribution against mosquitoes. ARS developed—and USDA patented in 2003—SS220, a repellent that's just as effective as DEET. (See "DOD Partners with ARS To Protect Troops From Insect Vectors," *Agricultural Research,* September 2005, p. 12.)

DEET, the world's most-used insect repellent, was itself developed by ARS for the U.S. Army decades ago.

"In laboratory tests, isolated callicarpenal was just as effective as SS220 in preventing mosquito bites," says Cantrell.

Those tests were conducted by Klun against the mosquito species *Aedes aegypti,* which is best known as the yellowfever mosquito, and *Anopheles stephensi,* which spreads malaria in Asia.

Klun used the same system he used to test SS220: a six-celled, in vitro bioassay he and colleagues developed that evaluates bite-deterrent properties of compounds intended for human use. It consists of mosquito-holding cells positioned over compound-treated cloth covering six blood-membrane wells. The number of insect bites through the cloth determines compound effectiveness.

Cantrell says a patent application has been submitted for callicarpenal. Subsequent work will include tests against ticks and developing ways of producing large quantities of the compound, either through synthesis or crops. Toxicity trials will precede any testing on humans.—By **Luis Pons,** ARS.

This research is part of Plant Biological and Molecular Processes (#302) and Quality and Utilization of Agricultural Products (#306), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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CHARLES T. BRYSON (D419-1)

Berries and leaves of American beautyberry, *Callicarpa americana,* on Pinedale Farm. The Mississippi farm was once owned by John Rives Crumpton, grandfather of ARS botanist Charles T. Bryson.

Frozen Flies Safeguard Research, Screwworm Eradication Efforts

Using cryopreservation techniques, ARS entomologists can now freeze screwworm fly embryos—to later thaw and rear them to adulthood. The advance should cut the costs of maintaining fly colonies for research or of programs using the sterile-insect technique.

Considered the backbone of screwworm eradication, sterile-insect releases have helped eliminate this parasitic fly species from the United States and Central America as far south as the Isthmus of Panama. In the approach, both male and female screwworms are reared under factorylike conditions and then sterilized by irradiation. The sterile insects are then released to mate with wild flies, resulting in no progeny. Eventually, the fly population collapses. (See "Squeezing Out Screwworm," *Agricultural Research,* April 2001, pp. 18-22.)

"Freezing embryos eliminates the need to continuously rear flies used in research or held in reserve as backup strains for the mass-rearing and release program. This is costly, especially if there's no immediate need for them," notes Roger Leopold, an entomologist with the Insect Genetics and Biochemistry Research Unit at ARS's Red River Valley Agricultural Research Center, Fargo, North Dakota. Leopold's efforts to cryopreserve fly embryos first began there in the mid-1980s, "but it didn't really get going until the early 1990s," he says. That's when he consistently began reviving frozen embryos of house flies and other species.

Around 1996, he teamed up with Dennis Berkebile, an entomologist specializing in screwworm genetics at ARS's Midwest Livestock Insects Research Unit, Lincoln, Nebraska. Along with two other collaborators—Harvey Blackburn, an animal geneticist at ARS's National Center for Genetic Resources Preservation, Fort Collins, Colorado; and Arun Rajamohan, a postdoctoral researcher at North Dakota State University—Leopold and Berkebile explored creating an embryo-based repository to back up colonies of 10 key screwworm strains. Up until August 2004, these strains were kept at ARS's Lincoln site and the screwworm mass-rearing plant in Tuxtla Gutierrez, Mexico. (In August, the Lincoln unit concluded its screwworm program with the transfer of some of its last remaining colonies and biocontainment equipment to a new facility being built in Panama.)

According to Berkebile, some of the Lincoln colonies date back 20 years and include strains with useful mutations, including colored eyes and curly wing deformities. Scientists prize such mutations because they can serve as markers to important genetic traits, like fitness, and can be used to create all-male strains of screwworm with potential to cut fly-rearing costs. Such strains could prove useful if screwworm eradication expands into South America and the Caribbean.

Berkebile says creating a repository addressed fears of losing the strains to calamities such as disease outbreaks, fire, or even earthquake damage to facilities in which the flies are kept—derailing years of research in support of programs to eradicate the parasite and prevent its reintroduction. "The idea behind using cryopreservation was that we'd be able to preserve genetic material encoded within the embryos' DNA," says Berkebile. "So, if something were to happen to the strains they're mass-producing in Mexico, for example, we'd have these strains in storage to bring back and start the colonies all over again." The alternative would be to capture wild flies in field traps, screen them, and breed them in hopes they would be genetically similar to the lost strain.

Embryo cryopreservation also offers a way to safeguard against unwelcome mutations and inadvertent selection. Both events can change a colony's unique genetic makeup, rendering it less useful to research or sterile-insect applications. "If there are any genetic changes (either positive or negative) in the live populations, we would have the original genetic material to refer to in evaluating that change," says Blackburn, who leads the Fort Collins center's National Animal Germplasm Program. "Having a genetic repository for insects would allow breeders to determine what genes may have been lost in the selection process."

So why not freeze adult flies, rather than the embryos, and thaw them out as needed? Because embryos lack a cuticle—the outer covering of an insect that protects it from water loss and environmental elements. This makes it possible for scientists to extract water from the embryo and replace it with a cryoprotectant like ethylene glycol, which prevents the embryo's 40,000 to 50,000 cells from being damaged by ice crystals during freezing.

"We're working with an insect stage that's comparable to a 7-month-old

SCOTT BAUER (D015-1)

To prepare screwworm eggs for penetration by a cryoprotectant, entomologist Dennis Berkebile and technician Staci Bohling remove the eggs' outer layer.

mammalian fetus—if you consider the stage of differentiation of the cells and organs," Leopold explains. Differentiation is the process whereby cells begin to form specific tissues and organs. Even with embryos, he adds, "Cryopreservation at this stage of differentiation is usually very difficult."

The process begins with collecting eggs soon after they're deposited by caged female flies. The eggs are incubated to the correct developmental stage; loaded with the cryoprotectant, which eliminates most of the cell water; and then "vitrified," meaning they are cooled so rapidly that the cryoprotectant and any remaining water turn to a glass rather than to ice crystals.

The scientists then plunge the embryos into liquid nitrogen (-196˚C) and store the vitrified eggs in metal cassettes within a tank of liquid nitrogen. According to Leopold, the eggs can be stored there indefinitely at a cost of pennies per day. Depending on the number of strains in culture, maintaining live caged colonies, by comparison, can cost thousands of dollars per month.

To revive the embryos, scientists remove the cassettes from liquid nitrogen, hold them in nitrogen vapor for a short time, and then immerse the embryos in fetal bovine serum, which removes the cryoprotectant. Thirty to 45 minutes later, the embryos begin moving within the eggs. About 8 to 12 hours later, they hatch as larvae. The hatchlings are then reared on an artificial diet of dried blood, egg, milk, and other ingredients that simulate their natural diet of animal flesh.

So far, the longest the scientists have stored the cryopreserved eggs is just over 1 year. But Leopold reckons these eggs, like embryos of other animals currently in storage, can last indefinitely in their frozen state. When he and Berkebile first tried this technique on two screwworm strains, about 30 to 40 percent of the eggs hatched after being frozen. Now, oftentimes 70 to 80 percent hatch, with 30 to 60 percent of the larvae developing into adult flies. The researchers expect to bolster that rate with a new, improved diet that they devised for recovering cryopreserved larvae.

Meanwhile, they've also begun storing cryopreserved embryos inside tanks at ARS's Fort Collins germplasm unit. Over the next couple years, they'll monitor the embryos' viability and use revived specimens to check for genetic changes in live colonies.

Historically, the focus of ARS's National Center for Genetic Resources Preservation has been to preserve germplasm from plants in the form of seeds or pollen. The focus has recently been expanded to include livestock and aquatic germplasm as frozen sperm and embryos.

"We see potential for expanding the program to include cryopreservation and conservation of insect genetic resources," says Blackburn. "For example, we've already had discussions with several labs about cryopreserving bee semen. Clearly, the spectrum is limitless for both harmful and beneficial insects."—By **Jan Suszkiw,** ARS.

This research is part of Veterinary, Medical, and Urban Entomology, an ARS National Program (#104) described on the World Wide Web at www.nps.ars.usda.gov.

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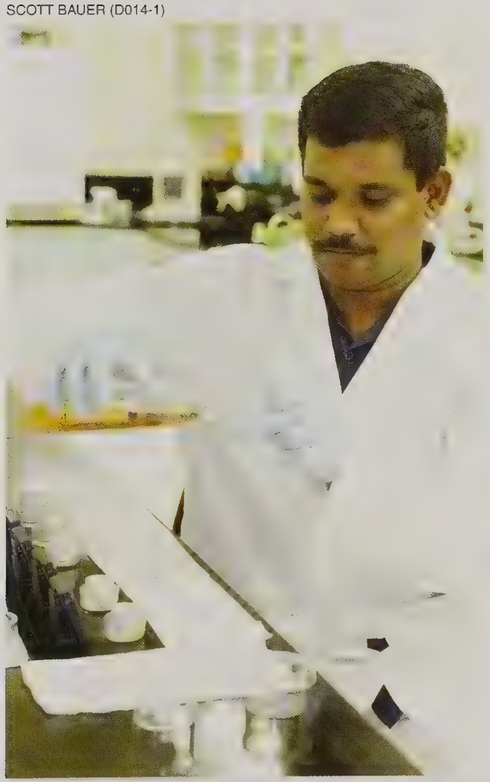
SCOTT BAUER (D014-1)



While visiting the Midwest Livestock Insects Research Unit in Lincoln, Nebraska, postdoctoral researcher Arun Rajamohan (from Fargo) prepares screwworm embryos with a cryoprotectant before storage.

SCOTT BAUER (D013-1)

In Lincoln, Nebraska, visiting entomologist Roger Leopold (from Fargo) places screwworm embryos in a liquid-nitrogen storage unit to be transported to the germplasm unit in Fort Collins, Colorado.

Improved Methods of Whitefly Control

Is there a way to control pest populations without abusing insecticides? Absolutely, say Agricultural Research Service (ARS) scientists in Maricopa, Arizona, and researchers from the University of Arizona.

The silverleaf whitefly, a common cotton pest, damages and kills crops by sucking their sap, spreading viruses, and excreting a sticky substance called honeydew. Spraying is a fairly effective control method, but some whiteflies are distressingly resistant, and many growers use costly chemicals conservatively. ARS scientists at the U.S. Arid-Land Agricultural Research Center are investigating ways to improve whitefly population management with minimal insecticide use.

Predator Identification

Biological control—reducing pest populations by using natural enemies—is one tactic that can replace or reduce spraying. Lab scientists developed a technique to identify predators by testing their guts for evidence of whitefly consumption. Using this method, scientists James Hagler and Steven Naranjo identified predation frequency for 18 whitefly predators, many of them previously unidentified.

Hagler developed the technique in the early 1990s, when whitefly populations were surging throughout the United States and scientists had little information on their predators. Using gut analysis, Hagler could screen more than 1,000 predators per day. This helped to quickly identify which ones should be conserved for optimal biological control.

The whitefly-specific ELISA (enzyme-linked immunosorbent assay) rapidly identifies natural predators. First, the suspected predator is crushed and placed on an ELISA plate. Proteins from the insect's guts bind to the plate. Next, the scientist adds a monoclonal antibody that binds only to whitefly proteins.

The scientist then adds a secondary antibody, which can only bind to the plate in the presence of the whitefly antibody. A catalyst is added to show whether binding has occurred. A color reaction indicates that it has, meaning the insect has eaten a whitefly. This procedure allows scientists to identify potential predators without disturbing the field's natural order with cages or other restrictions.

JACK DYKINGA (K4813-17)

Having glued a whitefly to a leaf, the big-eyed bug can devour its prey.

Predator-Prey Interaction

Understanding pest-predator interaction helps scientists develop more effective management practices. Hagler also uses ELISA to study pest and predator dispersal patterns by marking them with proteins. The insects are released, recaptured, and analyzed by protein-specific ELISAs. He has found proteins more reliable than traditional markers like paints, dyes, dusts, and trace elements.

In the first open-field study using protein to mark insects, Hagler and his colleagues tracked the dispersal of *Eretmocerus emiratus,* a parasitic wasp. More males were recovered than females, indicating different dispersal habits. That's important information for farmers who want optimum performance from biological control agents. Currently this technique is being adapted to study dispersal characteristics of termites, mosquitoes, honey bees, ants, and many other insects.

While gut analysis helps to identify predator species, it does not measure their impact. Naranjo and University of Arizona researcher Peter Ellsworth conducted extensive studies of how whiteflies die. By tracking individual immature whiteflies in the field, they identified common causes of death, such as predators, parasites, and dislodgement. They also discovered that whiteflies were most vulnerable to predation during the fourth nymphal stage. This led them to recommend conserving

JACK DYKINGA (K7637-7)

Entomologist James Hagler views results of an ELISA test. Bluish-colored wells indicate the presence of whitefly remains in the stomach of predator insects.

JACK DYKINGA (K7549-7)

A tiny pirate bug, *Oris insidiosus*, feeds on whitefly nymphs.

natural predators and targeting whiteflies during stage four.

Naranjo and Ellsworth found that natural death rates, though fairly high, are inadequate to reduce pest populations. According to their research, death rates of immature stages must exceed 98 percent to maintain a stable population, and reducing their ranks requires an even higher rate. Naranjo concluded that biological control alone is not enough to suppress whitefly populations. Moderate spraying may reduce pest numbers, but conventional insecticides can kill predator and prey alike.

So how do growers protect fields without harming the biological control agents they've enlisted?

Insect Growth Regulators

"Conservation of natural enemies by using selective insecticides is a major program component," says Naranjo. He and Hagler recommend complementing biological control with insect growth regulators. With Ellsworth, they studied the effects of growth regulators buprofezin and pyriproxyfen on 20 common predators. The researchers found that common insecticides reduced the population of all predator groups, whereas the growth regulators only reduced the densities of eight—and then at a lower rate.

Even within those reduced groups, the predator-to-prey ratio was higher with growth-regulator use, indicating that it poses a greater threat to whiteflies than to their enemies. Further mortality studies by Naranjo and Ellsworth confirmed that conservation through use of selective insecticides leads to higher predation rates. Hagler and Naranjo also used the gut analysis to monitor the sub-lethal effects of insecticides and growth regulators on predators' feeding habits, confirming that buprofezin and pyriproxyfen are gentler on most predators than conventional insecticides.

Research Benefits

ARS and University of Arizona research has contributed to more effective whitefly control, benefiting the growers, the public, and the environment. The scientists have developed a successful program for integrated pest management, giving cotton growers alternatives to insecticides. "We have shown that conservation biological control is possible, to a limited extent, if you know your key natural enemies and use selective insecticides," Hagler says. Naranjo agrees.

They attribute the program's success to pest-avoidance methods like biological control, careful insecticide use, and predator conservation. Their work is part of a growing knowledge base, helping decrease insecticide use for whiteflies by about 85 percent since 1995. Naranjo believes the whitefly management program is responsible for "a significant insecticide use reduction."

The research team's recommendations for preventive action, selective insecticides, and biological control have helped growers respond more effectively to pest invasions.

"The more we can exploit pests' natural enemies through conservation biological control, the less we have to rely on pesticides," Hagler says.—By **Laura McGinnis,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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STEPHEN AUSMUS (D288-4)

A pair of silverleaf whiteflies, *Bemisia argentifolii,* measuring one-tenth of an inch long.

ARS EXPERTS AS FIREFIGHTERS

Our scientists respond to international agricultural problems that affect the United States

Most of the time when you think of scientists solving problems, you picture months or years of experiments and study. But sometimes ARS scientists are called on to be firefighters—helping cool down international agricultural hot spots that are critical to the United States and the world.

On December 26, 2004, the freighter Yarmouth was stuck dockside in Béjaia, Algeria, unable to unload 18,600 tons of U.S. durum wheat because the Algerian government feared the shipment was infected with Karnal bunt, a contagious fungus that infects wheat.

Many countries require either immediate quarantine of wheat exports from areas reporting the presence of Karnal bunt or phytosanitary guarantees that the fungus is not present in a shipment. Several outbreaks have occurred in the United States since Karnal bunt was first reported here in 1996, but the last time was in 2001.

With a large but volatile wheat export market like Algeria, which can bounce from \$20 million to \$100 million a year, at stake, it was essential that the United States provide clear, scientific assurance that this shipload of wheat would not put Algerian agriculture at risk and that routine U.S. Karnal bunt precautions are strong enough to keep other countries safe.

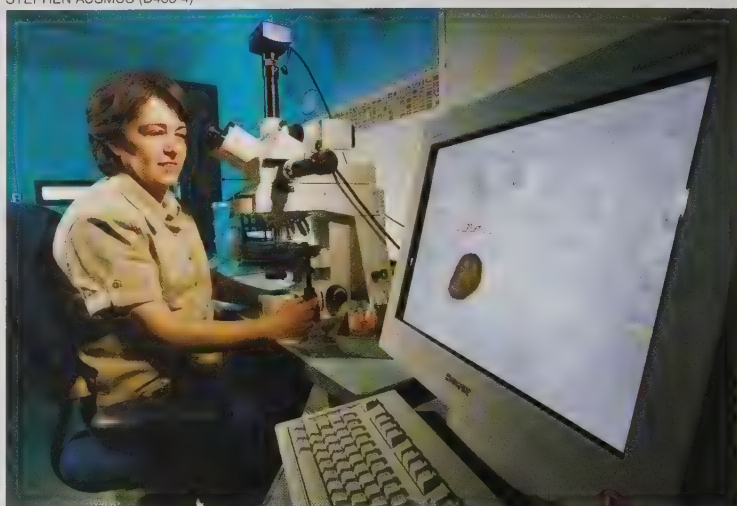
With time of the essence—it is costly for ships to idle in port, not to mention the damage to U.S. wheat's reputation—USDA's Foreign Agricultural Service (FAS) and Animal and Plant Health Inspection Service (APHIS) and Algerian authorities turned to ARS's leading Karnal bunt expert, mycologist Lisa Castlebury, with the Systematic Botany and Mycology Laboratory at the Beltsville (Maryland) Agricultural Research Center (BARC).

Castlebury's day-to-day job is researching the systematics of plant pathogenic fungi, especially the Tilletiales (bunt fungi) and Diaporthales (chestnut blight)—how these fungi operate biologically. Such basic information helps to accurately identify disease and quarantine pests and provides the foundation from which to develop effective disease-management strategies.

"Karnal bunt can be difficult to distinguish from a look-alike but harmless fungus usually associated with ryegrass," Castlebury explains. "They can be easily confused, especially in situations where only a few spores are present, and the result can be needless and costly quarantines."

But Castlebury is one of the scientists who described the ryegrass fungus and detailed features that distinguish it from Karnal bunt fungus. She also helped develop one of the tests used to distinguish the two.

STEPHEN AUSMUS (D483-4)



Mycologist Lisa Castlebury examines a digital image of *Epicoccum*, a commonly encountered Karnal bunt look-alike, in a seed wash from the U.S. wheat that was being held at the port in Algeria.

With hopes that she could convince that country's authorities and free the wheat shipment, Castlebury was flown to Algeria.

"I looked at samples and slides prepared by the Algerian quarantine scientists and met with the Algerian Secretary General of the Ministry of Agriculture and his experts, explaining the science behind my identification methods and just how reliable the information really was," Castlebury says.

On February 2, Algeria allowed the shipment to be unloaded.

"Dr. Castlebury's expertise was essential to solving the technical aspects of this problem," says Karen Z. Ackerman, APHIS phytosanitary manager/trade issue director for this region.

"Since Algeria is the world's largest durum wheat importer, restoring Algerian confidence in U.S. wheat supplies was extremely important to our wheat farmers," adds Michael J. Fay, the U.S. agricultural attaché, who coordinated the U.S. response.

LISA CASTLEBURY (D488-1)

Port facilities in Algiers.

J. MARIE METZ (D486-1)

Thrips palmi, **a crop-destroying insect (about 1.5 mm long) that can affect a wide variety of crops including sugarcane, beans, cantaloupe, melons, peppers, potatoes, and soybeans.**

Thrips Take a Trip—to Cuba

Sometimes it is not a country that an ARS scientist must convince, but rather world opinion. Such was the case in 1996, when Cuba accused the United States of releasing crop-destroying insects called "*Thrips palmi*" during an authorized overflight by a U.S. narcotics crop-eradication plane from Florida to Bogota, Colombia.

T. palmi, which can damage a wide variety of crops, including beans, cantaloupe, melons, peppers, potato, and soybeans, was first described in Indonesia in the 1920s.

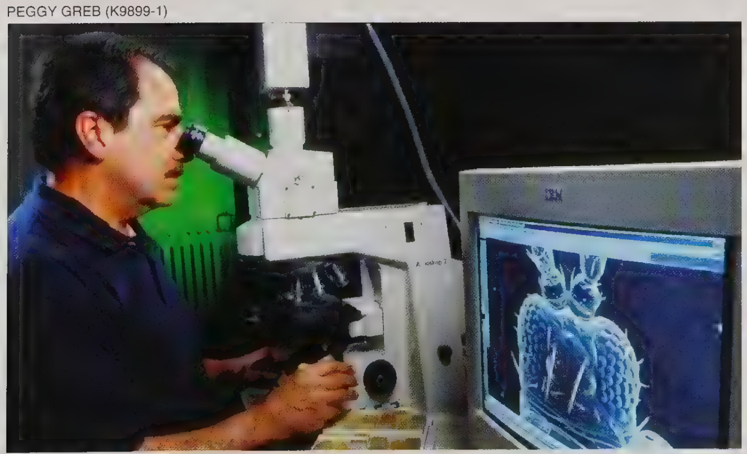
Cuba charged in the United Nations that the United States released these thrips from the plane, accidentally or deliberately, to spoil their sugarcane crop—an act of biological warfare. A Biological Weapons Convention consultative meeting/hearing was held in Geneva in August 1997.

There was never any question that at the end of 1996 *T. palmi* were being found in Cuba. But how did they get there?

To answer that question, the United States called on entomologist Sueo (Steve) Nakahara, now retired from ARS's Systematic Entomology Laboratory (SEL) at BARC. The lab is one of the foremost authorities on agronomic insect identification. Many countries submit samples to it and rely on the staff's expertise.

Nakahara began by checking the scientific literature for records of *T. palmi* in the Western Hemisphere. It was first found in Martinique in 1985 and reached Florida by the end of 1990. Then he combed the U.S National Collection for thrips that had been sent at various times from a variety of locations for identification by SEL.

PEGGY GREB (K9899-1)



Entomologist David Nickle, at the ARS Systematic Entomology Laboratory in Beltsville, Maryland, is one of the ARS specialists who identifies insects to help ensure safe and timely shipments at various ports of entry.

He combined that data with his expert knowledge of the behavior and spread of thrips and projected when a natural spread would have reached Cuba.

"When Steve compared his projected arrival time with when the Cubans reported finding the thrips, the thrips were right on schedule," explains SEL research leader M. Alma Solis. "That's part of the value of having a lab like SEL. Although we do basic research, we are the resource for information and studies that provide answers like the one needed here."

PEGGY GREB (K9809-1)

In efforts to control or eradicate avian influenza, veterinary medical officer David Suarez provides expertise and guidance to several Southeast Asian and eastern European countries to set up practical and effective laboratory systems.

Animal Disease Experts

The ARS Southeast Poultry Research Laboratory (SPRL) in Athens, Georgia, has taken on a special role in international efforts to deal with one of today's most pressing animal health issues: avian influenza (AI). In April 2005, the United Nations Food and Agriculture Organization and the World Organization for Animal Health launched the New Worldwide Avian Influenza Network (known as "OFFLU") to coordinate reference laboratories focusing on AI in animals and those focusing on human influenza. The network, of which SPRL is part, is designed to speed up exchange of scientific data among leading diagnostic and research institutions.

"ARS brings unique resources and facilities to the table, like being able to do rapid sequencing to identify changes in AI viruses and vaccine safety and efficacy studies," says David E. Swayne, director of the Athens lab. "We need to have the experts linked together if we are going to expand our scientific knowledge and improve efforts to control or eradicate AI around the world."

ARS AI experts have also been in demand by countries facing this critical issue. In particular, David L. Suarez, research leader of the Exotic and Emerging Avian Viral Diseases Research Unit in Athens, has been lending expertise to several Southeast Asian and eastern European countries.

"Funding has come from the United States, host countries, and others for me to help other countries set up practical, effective systems and provide guidance on what types of bird vaccines they need," says Suarez.

For example, in fall 2005, Romania asked Suarez to evaluate its laboratory capabilities and provide technical suggestions after they confirmed cases of H5N1 AI in birds there. In December 2005, he traveled to Vietnam to teach a course on real-time PCR diagnostic tests for AI strains—tests he helped develop.

Sensing Screwworm

While ARS's help with AI has been preventive, other cases have involved a more active role in solving an animal disease problem.

When Aruba, a tiny island in the Caribbean, had an outbreak of screwworm in March 2004, it contacted ARS remote-sensing specialist Pamela L. Phillips, who works with the Mexican-American Commission for the Eradication of Screwworm (COMEXA) from the ARS Screwworm Research Unit in Panama. ARS's invention of the sterile male insect release control method was the basis for eradication of screwworm in the United States in 1982.

ARS continues to research ways to eradicate screwworms, but in other countries rather than here, because screwworm has been eradicated south to the barrier zone in Panama.

JOHN KUCHARSKI (K7576-1)

Close-up of a screwworm larva. Tusklike mandibles protruding from its mouth tear the flesh of living warm-blooded animals. A wound may contain hundreds of the larvae.

Aruba knew it needed immediate help before the screwworm outbreak problem grew worse. Not only was the local livestock industry endangered, but there was concern for the country's number-one industry, tourism, because screwworms can cause untold devastation to all warm-blooded animals, including humans.

"Because Aruba is only 112 square kilometers and has high trade winds, we couldn't use our conventional dispersal method of aerial release of sterile male screwworm flies," Phillips explains. "But I had come up with a new method where I use satellite imagery to identify areas that would be favorable habitat to set out ground-release chambers for sterile screwworm pupae."

COMEXA agreed to try this new method for the first time, and the Aruban government officially asked the U.S. government for assistance.

THEODORUS WOOLS (D487-1)

Ground-release boxes of sterile male screwworms were used in 2004 to eradicate screwworms from Aruba.

First, Phillips identified the favorable habitats on LANDSAT satellite data, along with prevailing wind directions and locations where screwworm infested animals had been found.

"Then we stayed in contact with Aruban veterinary teams to monitor the pattern of positive screwworm cases and to identify additional areas for ground release as needed," Phillips says.

Ground releases began on June 7, 2004. "The last screwworm case in Aruba was reported on October 13, 2004, but we continued releasing sterile flies until December 16, three fly life cycles after the last positive find," Phillips recounts.

An Objective Voice

ARS scientists aren't always called on to travel to a foreign country to help cool off an international hot spot; sometimes others come to us.

When Brazil, the largest buyer of U.S. wheat in South America, banned its import in 2000 because of concerns about wheat seed gall nematode, FAS and APHIS turned to David Chitwood, zoologist and research leader with the ARS Nematology Laboratory at BARC.

These nematodes had been widely found in the United States until wheat cleaning—which easily and completely removes them—became customary. The last find was reported from Virginia in 1975.

But Brazil believed that the nematode was still present in the United States and didn't want it in Brazil.

"We had a team of Brazilian nematologists, plant pathologists, and an agricultural trade expert spend a week here looking at U.S. wheat for the nematode. The Federal Grain Inspection Service shipped in samples from all around the United States. Each wheat sample was found to be free of plant parasites," Chitwood says, who hosted along with ARS plant pathologist Lynn Carta.

What capped the controversy was when Chitwood and ARS microbiologist Zafar Handoo went through the U.S. Nematode Collection, which their lab oversees, and found a sample of a seed gall nematode collected from Brazil in the 1950s, proving that Brazil is already home to at least the same genus of problem nematodes. The identification couldn't be any more specific because the sample nematodes were juveniles.

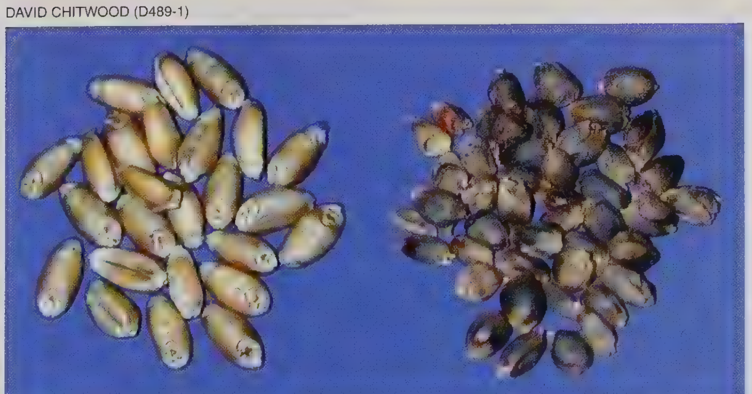
Brazil lifted the import ban in early 2001.

"They came to us because we have the most expertise in identifying nematode species," Chitwood explains. "They trusted ARS as an objective scientific voice. They felt comfortable that ARS, as a scientific, nonregulatory agency, doesn't have anything at stake in a trade issue."

Objective expertise was also what prompted the Maine Department of Agriculture to contact ARS chemist Robert Owens, with the Molecular Plant Pathology Laboratory at BARC, for help in the spring of 1996. A shipment of Maine seed potatoes was prohibited from being planted in New Brunswick, Canada, because it was believed to be infected with potato spindle tuber viroid.

"I knew this farmer and his operation and knew that he was working with clean stock from tissue culture, so I couldn't believe he would have seed infected with this serious pathogen," recounts Terry Bourgoin, director, division of plant industry, Maine Department of Agriculture. "Potato spindle viroid is not a quarantine issue for the United States, so APHIS wasn't involved. I needed an expert familiar with this pathogen to help us prove that the operation was clean and that the testing was the problem."

DAVID CHITWOOD (D489-1)



Healthy wheat (left) and wheat infected by wheat seed gall nematode.

STEPHEN AUSMUS (D482-3)

ARS chemist Robert Owens (right), microbiologist Susan Thompson, and technician John Clark review results of a "dot blot" assay for potato spindle tuber viroid. These assays provide sensitive, rapid detection of infected plants and identify any viroids present.

The crux of the matter was what type of test would be used to determine whether or not potato spindle tuber viroid was present. Ag Canada had certified several private laboratories to test RNA samples. A test had found a band of RNA that might have been potato spindle tuber viroid.

"We retested the potatoes using a much more sensitive and specific test for viroid nucleic acids and found no trace of potato spindle tuber viroid," Owens says.

Then Owens explained the efficacy of the test methods and his results to the Maine Department of Agriculture and Ag Canada.

"Ag Canada changed its decision because they saw ARS as an objective scientific authority, equal to their organization," Owens says.

Eventually, the grower received a financial settlement for his potatoes, which had been destroyed.

That fall, a meeting was organized by the Canadians with the United States and Mexico to discuss acceptable testing standards. Owens attended as a subject expert.

"It's not ARS's job to be the first responder when a crop shipment gets stopped at a border, but we are seen as a source of authoritative science," Owens says.

Bourgoin adds, "Without Bob's help, not only would that grower have been stuck for the financial loss of the seed potatoes, he would have been prohibited from shipping to Canada for several years, and Maine's whole seed potato export industry would have been in jeopardy."

The Cotton Concurrence

Quarantine and disease are not the only types of international fires ARS helps to extinguish.

An ARS team led by Xiaoliang "Leon" Cui, a cotton technologist, and Patricia Bel, a textile engineer, both at the ARS Southern Regional Research Center in New Orleans, Louisiana, ensured that U.S. cotton producers would continue to be able to sell their product to the People's Republic of China, a market worth about $733 million a year.

In 2002, China notified the World Trade Organization that it was going to institute new mandatory standards for short fiber content and white-speck nep count in cotton bales as measured by tests not currently used in international trade. "White-speck neps" are small knots of tangled fibers that can cause a dye defect and reduce fabric quality.

Since the new tests would be time-consuming and costly and would only be required in China, they represented a potential trade barrier. This concerned both USDA's Agricultural Marketing Service (AMS), which guides cotton quality standardization in the United States, and the National Cotton Council of America. So the two groups turned to ARS for help in evaluating the new China test before the new regulations would take effect. Cui is a leading expert in measuring cotton fiber length, and Bel is an expert in measuring neps.

But before the ARS scientists could even begin their evaluation of the new tests, they first had to obtain the test instrument that was going to be used by the Chinese. Then they had to translate the test standards from Chinese into English.

In just a matter of weeks, the ARS scientists finished experiments to see how reliable China's methods were. They also established equations using results from conventional laboratory fiber testing methods to predict the fabric quality results if Chinese cotton were tested by conventional U.S. methods and then compared to U.S. cottons.

Then Cui organized a briefing for Chinese cotton experts. He explained the current internationally recognized test methods, the reliability of those methods, and how they matched up with the proposed new tests. Armed with research results and technical advice from ARS, AMS was able to quickly respond to Chinese government officials regarding technical aspects of the new requirements.

The Chinese government then postponed implementation of the new standards and soon announced that the Chinese cotton classification system would be reformed. This reform will not only help China modernize its classing system, but will also facilitate export of U.S. cotton to that country.

"The ARS work was a big help to us, and we helped the Chinese adopt internationally recognized methods," says Norma R. McDill, the then AMS deputy administrator for the agency's cotton program. "Since U.S. cotton exports to China are about 5 million bales a year, this was an important accomplishment."

NATIONAL COTTON COUNCIL (D491-1)

ARS scientists helped preserve the U.S. cotton export market to China by helping their industry understand U.S. quality standards.

"The fast work and expertise of the ARS scientists were critical to gaining Chinese officials' commitment to internationally recognized standards and testing methods that are important to the U.S. cotton industry," adds Andrew G. Jordan, vice president for technical services for the National Cotton Council of America.

With ARS's depth and breadth of expertise, it will no doubt continue to be called on to respond when foreign agricultural fires flare up.—By **J. Kim Kaplan,** ARS.

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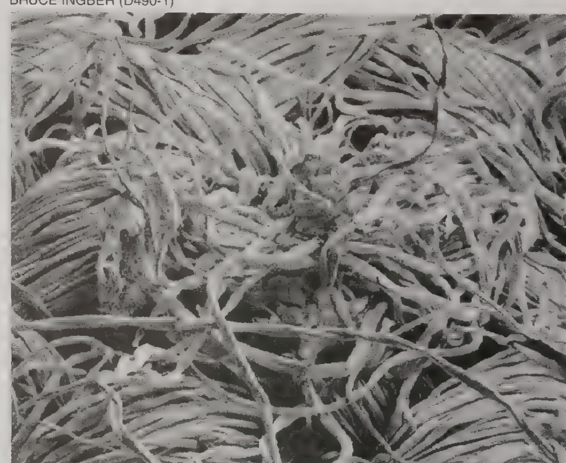
BRUCE INGBER (D490-1)



Scanning electron micrograph of a cotton white-speck nep, which is a small knot of tangled fibers that can cause a dye defect.

PEGGY GREB (D269-1)

BeetleMania

With its genes recently deciphered, an ordinary beetle goes from lowly pest to scientific star.

A shiny, rust-colored beetle, only 1/8-inch long, scuttles across a kitchen countertop. It can't resist the nearby stash of cupboard items for which members of its species have such an affinity—cornmeal, wheat flour, cake mix, crackers, and chocolate.

The pesky bug might seem nothing more than a nuisance to most. But to ARS entomologist Richard Beeman, this everyday pantry invader—the red flour beetle—has been the focus of more than 20 years of research.

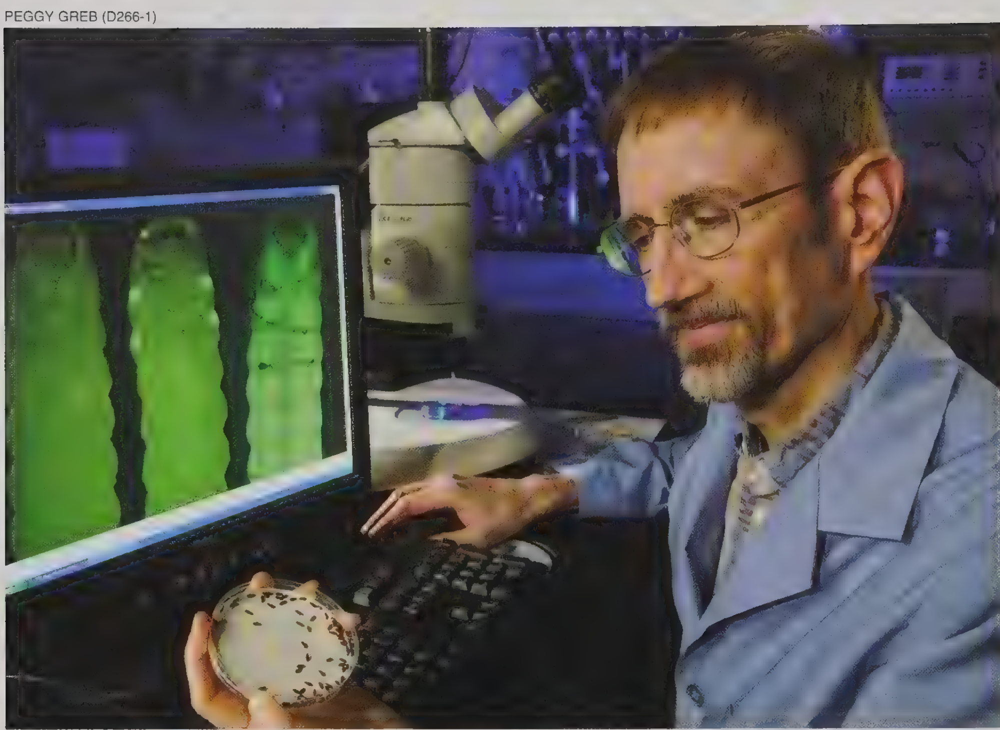
Beeman, who works at the ARS Grain Marketing and Production Research Center in Manhattan, Kansas, has spent his career delving into the genes of *Tribolium castaneum*, hoping to unlock important secrets of this costly agricultural pest, its beetle cousins, insects as a group—and even ourselves.

Expensive Tastes

With their voracious appetite for stored cereals and nuts, the red flour beetle and its kin cause millions of dollars of damage annually. They can infest food products at any stage—from raw grain to finished baked

Due to a defect in their pigment gene, the red flour beetles above have white or clear eyes rather than the typical black color. Other defective genes are being discovered and exploited as possible biopesticide targets.

PEGGY GREB (D266-1)



The glowing-green beetle larvae shown on the monitor have been tagged with a fluorescent marker gene that attaches to different genes within the larvae. This technique helps entomologist Richard Beeman better understand the function of new genes identified through genetic sequencing of the beetle.

Plus, *Tribolium* stands out among insects as a generalist feeder that is very capable of surviving by feeding on a wide spectrum of foods. "It's not specialized like the fruit fly, which feeds narrowly on rotting fruit," says Beeman, "or the honey bee, which feeds exclusively on pollen and nectar."

The beetle's diet is actually more like our own. Before humans began stockpiling grains on farms, inside warehouses, and in cargo ships, beetles were probably exploiting rich caches of grains and nuts inside mouse nests, Beeman thinks. "They may have been feeding on stored grains and nuts even before we were."

The beetle's also unusual in its ability to live in arid environments, possessing well-developed kidneylike organs to facilitate water retention. "As comparative knowledge of animal genomes progresses," Beeman says, "it's become increasingly

goods. And when storage practices, like warehouse sanitation, fail to suppress soaring populations, insecticidal sprays and fumigants are often needed.

The resilient beetle has sidestepped most chemical control efforts—even developing thousandfold resistance to one common pesticide, malathion. But instead of developing new insecticides, which face strict regulation, Beeman envisions a more ecofriendly approach.

PEGGY GREB (D268-1)

Normal red flour beetle (about 1/8-inch long) with black eyes.

By finding the soft spots in the insect's genetic armor, researchers may someday develop specific, genetically based ways of fending off the beetles. For instance, Beeman says wheat plants could someday be endowed with genes that disrupt the beetles' normal functioning, were they to take a bite.

In Proper Sequence

Beeman isn't the only researcher intrigued by the red flour beetle and its DNA. Two years ago, he and colleagues Susan Brown and Robin Denell at Kansas State University in Manhattan lobbied to have the beetle's entire genetic makeup decoded.

As they saw it, the beetle would be the first agricultural pest—and beetle—to have its genome sequenced. Its genes might then inform researchers about other organisms—filling current gaps in the evolutionary biology of the fruit fly and honey bee genomes, for instance.

PEGGY GREB (D264-1)

Technician Sue Haas uses a suction probe to remove flour beetles from a rearing jar containing flour. Beetles are reared in the lab for genetic testing.

clear that insects and humans share many common genes involved in metabolism, development, and immunity to infection."

Beetlejuice

In 2003, the red flour beetle was officially added to the growing queue of to-be-sequenced organisms. Before the project could begin, researchers needed starting material: copious amounts of the beetle's DNA. But from where?

Crushed-up beetle eggs, of course. Beeman was the likely candidate for supplying this vital component, since his lab was already home to thousands of beetles. Some possess a variety of genetic kinks, or mutations, that he's studying to learn more about the true functions of *Tribolium* genes.

Starting with a healthy red flour beetle from a corn bin in Georgia and subjecting it to 2 years of intensive breeding, Beeman and colleagues produced an army of identical beetle twins. About 100,000 of them were enlisted to turn out some 2 million eggs—about 50 grams worth. "It was a highly inbred strain," says Beeman. "To simplify interpretation of the genome sequence, we didn't want much variability across the beetle's individual genes." The eggs were frozen in liquid nitrogen and sent to Baylor College of Medicine's Human Genome Sequencing Center (HGSC), where their DNA was extracted and sequenced.

The first version of the genome sequence was released in January 2005 by HGSC. The final version will be released soon, and Beeman says the information it yields should prove to be a gold mine. With the sequencing data as their guide, he and ARS molecular biologist Marce Lorenzen and colleagues hope to identify all of the beetle's 15,000 genes.

Too Thin-Skinned?

Beeman has already tapped the unraveling genome to obtain basic information about how *Tribolium* and other insects produce the stiff outer coat, or cuticle, that's the hallmark of most invertebrates.

"Because the cuticle isn't elastic," he says, "insects get squeezed as they grow. Eventually they shed it and secrete another that's a bit larger—like getting a loose-fitting suit with growing room."

For several years, scientists have been trying to pinpoint genes that regulate the complex process by which insects develop—and shed—these outer shells, which are made of a substance called chitin. By "knocking out," or inactivating, candidate genes that genomic data had pointed to, Beeman and colleagues were able to isolate those responsible for orchestrating the chitin-production process.

PEGGY GREB (D265-1)

Molecular biologist Marce Lorenzen (left) and Richard Beeman review the recently developed genomic map of the red flour beetle.

"We got dramatic results," he says. "When we knocked out *Tribolium* genes that encode enzymes suspected to be important for cuticle tanning [hardening and darkening], the cuticle in those beetles just stayed white and soft." Without the genetic messages needed to tell their bodies to produce the right enzymes, the beetles simply died.

"Studies such as these will lead to better understanding of insect growth and development," says Beeman, "and provide better approaches for disrupting specific genes as a form of nonchemical insect pest control."

Beeman will continue to probe the beetle's DNA for more answers. A gene holding his interest for more than 13 years is one he discovered in the female that mysteriously causes some of her hatchlings to abruptly die, while having no effect on her. But offspring inheriting a copy of the gene survive.

Considered a maternally acting, "selfish" gene, it's called the *Medea* factor—named after the enchantress in Greek mythology who murders her own children for personal gain. Since Beeman's find, a similar gene has only been found in mice. "Medea's fascinating and unique," he says. "I want to learn more about its evolutionary origins and physiological mechanism."

As interpretation of the *Tribolium* genome continues, we'll likely learn more about that curious gene—and how we may share much more with the modest red flour beetle than just our appetite for grains.—By **Erin Peabody,** ARS.

This research is part of Plant, Microbial, and Insect Genetic Resources, Genomics, and Genetic Improvement, an ARS National Program (#301) described on the World Wide Web at www.nps.ars.usda.gov.

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A buck feeds from a plastic 4-poster. The design of the device causes the buck to tilt its head toward the application rollers, ensuring that Tickicide is transferred to its head, neck, and ears.

WAYNE RYAN (D513-1)

The Continuing Fight Against Cattle Ticks

Tick fever once crippled the U.S. cattle industry. The disease was eradicated here in 1943, but ticks that transmit it are still found in Mexico. Keeping them from crossing the border requires constant vigilance, with strict inspection standards and pesticide treatment of all live cattle imported from Mexico.

The southern cattle tick, *Boophilus microplus,* and the cattle-fever tick, *B. annulatus,* transmit the two species of blood parasites, *Babesia bovis* and *B. bigemina*, that cause the cattle diseases collectively known as "Texas fever," "cattle fever," or "bovine babesiosis." Texas fever can kill up to 90 percent of yearling and adult cattle. Cattle infected as calves with *Babesia* normally do not develop debilitating disease but may be less healthy than uninfected animals.

In 1938, as part of the USDA Cattle Fever Tick Eradication Program (CFTEP), a permanent quarantine area, or buffer zone, was established in southern Texas in a narrow, 500-mile-long area along the Rio Grande River. When outbreaks occur in the quarantined area, ticks are eradicated by CFTEP inspectors in cooperation with the Texas Animal Health Commission.

For almost 49 years, the ARS Knipling-Bushland Livestock Insects Research Laboratory in Kerrville, Texas, along with its satellite worksite at Moore Field in southern Texas, has been charged with providing technical support to CFTEP.

Wildlife Carriers

Since the buffer zone was created, the number of fever-tick introductions there has varied from year to year, but a significant increase has occurred over the past 5 years. In addition, an alarmingly large increase in the numbers of fever-tick infestations in the so-called tick-free area north of the buffer zone has been recorded in recent years.

Prime tick carriers are the hoofed, or ungulate, wildlife species, which abound in southern Texas and northern Mexico. White-tailed deer, American elk, and European red deer are all known carriers of *B. microplus* and *B. annulatus* ticks. Adding to the problem is a great increase, over the past few decades, in importation of exotic wildlife, which has brought hundreds of thousands of other animals, representing at least 67 species, into Texas.

In southern Texas, white-tailed deer are the most important alternative hosts for cattle fever ticks. In 2004, deer were associated with *B. annulatus* infestations of a cluster of ranches in the tick-free area of Kinney County, Texas. In the buffer zone on a ranch in Starr County, 19 of 25 white-tailed deer captured were infested with *B. microplus*. Similar incidents occurred in 2005 in Maverick County and in 2006 in Webb County in which ticks were found on cattle after being found earlier on deer.

A Simple Design Does the Trick

To combat spread of ticks by wildlife, Kerrville scientists developed and patented a device they call a "4-poster." It has a bin in the center from which small amounts of whole-kernel corn enter feeding ports located near each end of the device. When a deer inserts its muzzle into a port to feed, it must tilt its head and neck in a way that forces contact with a pair of paint rollers

saturated with pesticide. Later, when the deer grooms itself, the pesticide spreads enough to protect its entire body.

Initially created to combat the lone star tick, *Amblyomma americanum,* and the deer tick, *Ixodes scapularis*, which spreads Lyme disease, the 4-poster is now being tested for use against cattle fever ticks on deer.

Mat Pound, a Kerrville entomologist, tested a specially formulated acaricide, containing 10 percent permethrin, to treat tick-infested white-tailed deer. Pound's data were essential to gaining approval by the U.S. Environmental Protection Agency to market the product, named "Tickicide," to be used only with the 4-poster. Initial results indicate that the 4-poster will be useful for controlling cattle fever ticks on white-tailed deer.

Another technology developed at the Kerrville laboratory is a method of feeding deer whole-kernel corn treated with ivermectin or other systemic acaricides. It has often been used on ranches to eradicate ticks on deer once the cattle have been moved to another location.

"Use of ivermectin-treated corn and 4-poster technology is vital to the ongoing campaign to eradicate cattle-fever ticks introduced to southern Texas on hosts such as cattle, horses, white-tailed deer, or exotic ungulate wildlife that come across the Rio Grande from Mexico," says entomologist John George, who leads the research at Kerrville. "By using all our resources and knowledge, it will be possible to minimize or eliminate ticks on white-tailed deer and other ungulate wildlife, keeping U.S. cattle free of Texas fever."

DIANE KAMMLAH (D515-1)

A quarantine line was established in 1943 to create a buffer zone along the Texas/Mexico border, from Del Rio to Brownsville, to protect the southern United States against spread of ticks from Mexico.

SCOTT BAUER (K5442-8)

Cattle going through a tick treatment bath at McAllen, Texas. Scientists at Kerrville are working to find alternatives to these baths, typically used by ranchers.

New Ways To Beat Resistance

An important factor working against eradication is that some populations of southern cattle ticks in Mexico have evolved resistance to acaricides, and those resistant strains are spreading into southern Texas.

Boophilus tick control on U.S. cattle relies exclusively on treating the livestock with coumaphos, an organophosphate acaricide. But a recent ban on many organophosphate chemicals—plus a growing prevalence of resistance in many Mexican tick populations to organophosphates—is creating a critical need for effective alternatives.

At the Moore Field laboratory, researchers have tested a new formulation that uses spinosad, which is derived from a soil organism. They sprayed the spinosad, at 3-week intervals, all over *B. annulatus*-infested cattle, using two different concentrations—0.05 percent and 0.08 percent.

For comparison, untreated cattle were kept in a pasture beside the one with the treated animals, and tick counts were done at various intervals. In the pasture with treated cattle, the tick population declined, while ticks on untreated cattle increased. This suggests that spinosad application would be a useful addition to a tick-control program.—By **Alfredo Flores,** ARS.

This research is part of Veterinary, Medical, and Urban Entomology, an ARS National Program (#104) described on the World Wide Web at www.nps.ars.usda.gov.

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ROBERTA DUHAIME (D514-1)

The Rio Grande from a Border Patrol helicopter near Del Rio, Texas. The United States is to the left and Mexico is to the right. In many places, the river is shallow and can be easily crossed by animals that may carry ticks.

Beeting Back the Enemy

A chemical-free way to guard sugar beets against a vile pest

Technician Rob Schlothauer collects sugar beet samples in a field near the Sidney Sugars processing facility at Sidney, Montana. The samples will be examined for sugar beet root maggot damage.

BETH REDLIN (D591-2)

In his eastern Montana laboratory, Stefan Jaronski is focused on a cluster of green-glowing strands crisscrossing under his microscope. He's especially intrigued by the corkscrewing ones that appear to be choking off neighboring threads, like exotic vines spiraling around a tree.

Jaronski's subjects are dozens of soil fungi and bacteria—some of which he's endowed with a fluorescent protein from jellyfish so that they'll be easy to spot. Certain ones appear fairly passive, content to just hang out. Others reach out with armlike hyphae to rob a neighbor of its food. Even on this minute scale, the world is a cutthroat place.

An insect pathologist with the Agricultural Research Service, Jaronski is studying these minute interactions to find out who's most compatible. Which fungi and bacteria in the bunch are aggressors? Which are overly passive and vulnerable?

Just how these microbes mingle is significant because it helps Jaronski—who works at the agency's Northern Plains Agricultural Research Laboratory in Sidney—determine which of his top-performing fungi are best suited for the task at hand.

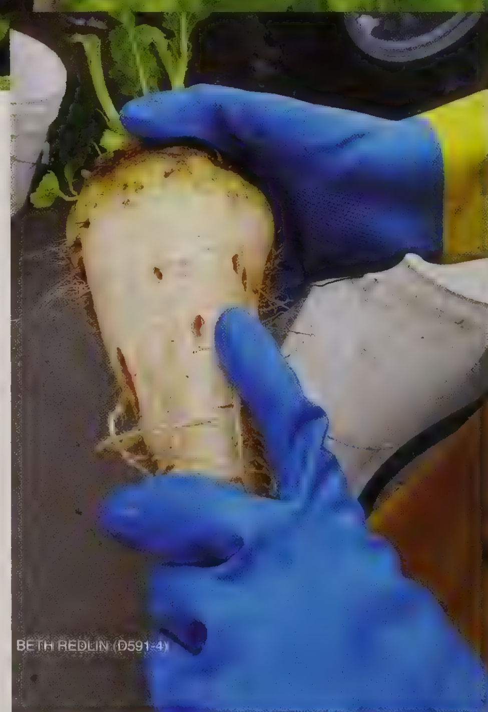
His challenge? Finding an all-natural way to protect sugar beets from a costly enemy in the soil. Beets supply the country with half its refined sugar, but more than 40 percent of U.S. fields planted with these sucrose-storing roots are plagued with a vile underground pest: the sugar beet root maggot.

STEFAN JARONSKI (D591-1)

The fungus *Metarhizium anisopliae* sporulating on a sugar beet root maggot.

A Tiny Terror: *Tetanops*

The root maggot is the wriggling, immature form of the tiny fly *Tetanops myopaeformis*. It lurks in the soil, gnawing on young sugar beet roots. This scarring weakens or kills the plants by exposing their tender tissues to disease-causing pathogens. The maggot's destructive feeding habits make it the most important



BETH REDLIN (D591-4)

Root maggot damage on a sugar beet root.

pest on the 1.4 million acres of sugar beets grown in the western United States.

Up to now, farmers have kept the maggots at bay by using a cocktail of insecticides. "In 2001, about half of U.S. sugar beet acres were treated with chemical insecticides to control this insect," says Jaronski. Because of the chemicals' potential toxicity, farm workers must be specially certified to apply them.

Jaronski thinks there are viable pesticide alternatives to be found in nature. "The soil is already chock-full of organisms ready to parasitize each other for food and survival." He'd like to take advantage of this rife competition by pitting one enemy against another. But which is the most potent against the root maggot? And which has the best chance of surviving in the microbial mix that makes up soil?

A Fungus That Fits In

The beneficial fungi that Jaronski is most interested in for the job are strains of *Metarhizium anisopliae*. Found all over the world, these soil dwellers are natural enemies of many insects.

"I like to think of *Metarhizium* fungi as the fatal 'athlete's foot' of insects," Jaronski explains. "Its spores are like those of bread molds and will germinate when they land on the surface of an insect." In about 24 hours, the spores penetrate the insect's skin, flourishing inside their host until they finally overwhelm it.

Jaronski wants to introduce these helpful fungi into beet fields suffering from maggot infestations. But for his plan to work, the fungi must be able to stand up to hundreds of other fungi and bacteria already residing in the soil.

Cindy Fuller-Schaefer, an ARS postdoctoral associate working with Jaronski, knows just how dynamic the world of soil fungi and bacteria can be. She worked for 9 months to make *Metarhizium* the "glow-in-the-dark fungi" that she and Jaronski could study easily.

She's watched them interact with other microorganisms that inhabit sugar beet roots—and it isn't always pretty. "If a fungus tries to invade a microbe's space, the microbe makes toxins to defend itself—and vice versa," says Fuller-Schaefer. "Fungi and bacteria can also exclude a competitor by stealing its nutrients. It's basically biological warfare."

In addition to surviving attacks from soil microbes, a fungus used as a natural control agent will need to take well to its sugar beet root environment. Just how vigorously the fungus grows and establishes itself determines how strong a barrier it will make.

The Most Promising Candidate

Currently Jaronski is focused on a *Metarhizium* strain called "F52." It's already registered in the United States for use against other insects, and it can be easily mass-produced, unlike other beneficial fungi.

"It's important to select the right *Metarhizium* strain," he says. "There are not only differences in the vulnerability of various fungi species to various soil microbes, but also in sensitivity among strains of the same species."

But so far, F52 is still the leading candidate, having passed all Jaronski's critical tests. "It looks like only a few soil bacteria can really affect its ability to grow," he says.

Field trials in Sidney since 2001 have shown that *Metarhizium* is capable of controlling the pesky maggot. The fungus's natural inhibiting abilities, Jaronski says, can be equal to the effectiveness of a powerful commercial insecticide.

Jaronski foresees delivering the fungus to fields in a granular form at the time of sugar beet planting. That way, it could colonize young plant roots and build up a protective barrier that incoming maggots couldn't penetrate.

He's also exploring a two-pronged biological control package for especially intense maggot infestations. "When maggots reach biblical proportions," says Jaronski, "as they've been known to do in northeastern North Dakota, a single approach is insufficient."

Along with collaborator Mark Boetel at North Dakota State University in Fargo, he's found that integrating *Metarhizium* with a small-grain cover crop—such as rye or oats—increases the fungus's effectiveness. The cover crop's dense, leafy foliage confuses the female *Tetanops* fly when she's searching for a place to lay her eggs. Plus, it provides a much-needed windbreak for young, struggling sugar beets.

Once he fully understands *Metarhizium*'s capacity to attack maggots and colonize sugar beet roots, Jaronski will focus on how to bond its spores onto a typical commercial seed.—By **Erin Peabody,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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CINDY FULLER-SCHAEFER (D591-3)

A microscopic image of the beneficial fungus *Metarhizium anisopliae* exhibiting a fluorescent green protein as it grows on the surface of a young sugar beet root. The fluorescence allows scientists to see how the fungus interacts with other microorganisms sharing the soil.

For Innovative Pest Control, a New Gene-Transfer Technique

GREG ALLEN (D596-1)

Insect physiologist Paul Shirk (left) and technician Richard Furlong examine a genetically transformed Indian meal moth caterpillar using fluorescence microscopy.

Many crop insecticides have lost effectiveness because the target pests have developed resistance. Others have been discontinued because of the potential or real risks they pose to environmental or human health. The upshot is that growers have fewer chemical options available to them for controlling pest insects.

One highly successful nonchemical alternative to insecticides is the sterile insect technique (SIT), in which masses of male insects are sterilized by irradiation and then released to mate with wild females. No offspring result, and the population diminishes.

SIT could potentially be improved—and new types of biological control developed—by use of genetically modified insects. A genetic tool developed and patented in part by ARS researchers, called the "*piggyBac* transposon," is being used to genetically modify many species of pest insects, such as flies, moths, and mosquitoes. Transposons are also called "jumping genes" because they can jump from one place on a chromosome to another. A concern about this genetic modification method, though, is the rare possibility that inserted genes could move again, or "remobilize," resulting in loss of the desired traits or entry into other organisms.

Before such modified insects are ever tested or used in the field, scientists must be sure any introduced genes stay put.

In an exciting discovery, ARS physiologist Paul D. Shirk and his colleagues at the ARS Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida, have developed a gene-transfer system—a "vector"—that can be used to test foreign gene activity in pest insects without worry that the genetic transformation will pass to the next generation.

This new method uses part of a virus called "*Jc*DNV" to ferry new genes into insects. The process is done by injecting insect embryos with the *Jc*DNV vector, which has been altered so it can't cause disease. Instead, the vector—carrying the foreign gene—inserts itself into the insect's chromosomes. The newly installed gene can then be tested during all stages of the insect's life.

Unlike *piggyBac* or other transposons, the *Jc*DNV vector enters only the somatic, or body, cells—not the germline, or reproductive, cells—so the next generation will not inherit the genes.

The vector works well for a variety of insects, including flies and moths. It allows testing of lab-constructed foreign genes that could potentially cause sterility in the insect, and there's no worry that the genes will remobilize.

The *Jc*DNV vector came from a parvovirus, *Junonia coenia densovirus,* which infects the common buckeye butterfly, *Junonia coenia.* Max Bergoin, a virologist who originally cloned the *Jc*DNV parvovirus in his laboratory at the University of Montpellier in France, collaborated with Shirk in the vector research. Other team members included postdoctoral researchers Herve Bossin and Jennifer Gillett and ARS technician Richard Furlong.—By **Jim Core,** formerly with ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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Pioneering ARS Entomologist

Since the establishment of Agricultural Research Service (ARS) in 1953, one of the most prominent of all USDA researchers was the late Edward F. Knipling. A world-renowned entomologist, Knipling developed pesticide-free ways to protect livestock and crops from the devastating effects of insects. He retired from USDA's Agricultural Research Service in 1973 after 42 years with the Department and continued to work with ARS as a collaborator after that.

Working with ARS colleague Raymond C. Bushland, Knipling pioneered the sterile male insect technique to suppress insect pests. This technique involves irradiating male insects, then turning them loose to mate with wild fertile female insects. These matings do not produce fertilized eggs, so numbers of insect offspring plummet dramatically.

Knipling and Bushland first developed the technique to combat screwworm flies, whose flesh-eating maggots parasitize livestock, wildlife, and humans. The technique resulted in the eradication of the wild screwworm population in the United States, Mexico, and parts of Central America, saving the North American livestock industry millions of dollars annually and winning praise from environmentalists.

SCOTT BAUER (K4722-2)

Entomologist Edward F. Knipling

Today, Knipling's technique is used worldwide to eradicate outbreaks of other pests such as Mediterranean fruit flies. In Africa, the technique is used to control the tsetse fly, which spreads sleeping sickness.

Knipling also is considered the founding father of the concept of areawide integrated pest management. Realizing that total eradication for most pests is not feasible, in the early 1980s Knipling developed the concept of using specific insect parasites, predators, and other tactics over a broad area to keep pest populations below the point where they impose a financial burden on farmers and ranchers. Kept at low levels, the pests would be more responsive to biological rather than chemical control.

Today, Knipling's areawide concept has grown to include not only parasites and predators as weapons against crop pests but also other environmentally friendly tactics, such as mating disruption and insect attractants.

For his numerous contributions to science and agriculture, Knipling won praise, awards, and tributes from many sources worldwide. In November 1999, *Progressive Farmer* magazine named him among 21 scientific pioneers who most shaped American agriculture in the past 100 years.

In 1995, Knipling was awarded the prestigious Japan Prize from the Science and Technology Foundation of Japan and was honored at a state dinner hosted by the Emperor of Japan.

His other awards include the National Medal of Science in 1996, the President's Award for Distinguished Federal Civilian Service in 1971, and the USDA Distinguished Service Award in 1960.

In 1967, President Johnson awarded him the National Medal of Science, the Nation's highest recognition for contributions to science. In 1966, Knipling was selected by Princeton University for the Rockefeller Public Service Award for distinguished public service in the field of science. In 1986, he was inducted into the ARS Science Hall of Fame for his research on the sterile insect technique and other technologies to suppress and manage insect pests.

Knipling began his career with USDA as a field aide in Mexico studying bollworms. Later, while on assignments in Iowa, Georgia, and Texas, he conducted research on various pests of livestock. From 1953 to 1971, he was the director of USDA's entomology division. During World War II, he worked on developing insecticides and repellents for the military. In 1971, he was appointed science advisor for ARS.

Edward F. Knipling was born in Port Lavaca, Texas, where he worked on his father's farm. He graduated from Texas A&M University at College Station and received a master's degree and doctorate in entomology from Iowa State University at Ames.

Adapted from an ARS daily news feed published online
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Entomology Research in the Federal Government

Past, Present, and Future

Past

Entomology had its beginning in the federal government when Townend Glover, an entomologist, was appointed on June 14, 1854, in the Agricultural Division of the Patent Office, Department of the Interior, to collect statistics on seeds, fruits, and insects. The Patent Office had become part of the newly established Department of the Interior on March 3, 1849. Federal entomology remained in the Agricultural Division of the Patent Office of the U.S. Department of the Interior from 1854 to 1862.

The United States Department of Agriculture (USDA) was established in 1862, and the responsibilities of the Agricultural Division of the Patent Office were transferred to the new Department.

The science of entomology has expanded in stature and numbers of subject areas since its inception in the USDA in research, regulatory, and pest management functions and has been extended into other branches of government. The first federal entomologist received his appointment as an "expert for collecting statistics and other information on seeds, fruits, and insects in the United States."

The first Commissioner of Agriculture formed the Division of Entomology with Glover as federal entomologist. Glover's natural history collections accomplished during his work in the U.S. Patent Office formed the base for an Agricultural Museum, established on August 1, 1864. The insect collections of the Agricultural Museum were transferred to the Smithsonian Institution in 1881. Early work of the Entomology Division consisted of species identifications, life history studies, and development of control methods. Recognition of the adverse impacts of insects on food and fiber production and relationships to human health were landmarks of entomological progress.

By the early 1900s, 35 entomological field laboratories were established in various states throughout the country. Entomological research had expanded to pursue knowledge of and control measures for major insect crop pests, some of which were insect vectors of plant diseases. The importance of entomological research was evident when mosquito-borne agents causing malaria, yellow and dengue fevers, and filariasis and tick-borne agents of tick fever of cattle were identified in the late 1800s and early 1900s. The emergencies of World War I revealed to the public the importance of insects and established entomology as a great science contributing to phenomenal increases in crop production when the war forced the United States to feed its civilians, troops, and allies.

The need for fundamental entomology studies in physiology and toxicology was recognized in the early 1900s, and authority was given to scientists to develop research without restrictions of focus on a specific pest or commodity. The concept prevailed and, although many USDA laboratories became established to address specific problems, pioneering research was supported. Pioneering research was not aimed at specific practical problems or objectives but rather at the advancement of science. The research sought to discover the underlying principles and to develop theory that would greatly facilitate problem-solving as needs arose.

The goal was to build a foundation for providing quick, effective, economic solutions to agricultural problems. The first official pioneering research laboratory was established at Beltsville, Maryland, on August 21, 1957, to investigate mineral nutrition of plants.

Sixteen pioneering research laboratories were established by 1961. Some of the research areas addressed were insect physiology, behavior, biological control, and insect pathology.

During World War II, insect control research in the federal agencies was a vital part of the war effort. High priority was given to protecting plants from insect ravages and increasing crop production of food and fiber to support the nation, its soldiers, and its allies. Control of lice, fleas, mosquitoes, and flies was vital to the health and operational efficiency of the military. Better methods and improved materials for delousing personnel and facilities were credited with reducing the incidence of typhus in the battle areas of Europe.

The development of aerosol insecticides, which were much sought after but proved difficult to make, was a major breakthrough in 1941 with the use of liquefied gas (Freon) as an insecticide solvent and propellant. The technology was immediately adopted and implemented in the war. Now, aerosol technology reaches into the lives of people throughout the world in its many applications in medicine, cosmetics, lubricants, household items, and numerous other applications. The full impact of the discovery remains grossly understated.

Other well-known findings during World War II include the discovery of hydrocarbon insecticide chemistry, insect repellants, and the role of DDT and its impact on facilitating the war efforts in the Pacific theater of war. On the home front, the Bureau of Entomology and Plant Quality (BEPQ), in cooperation with the War Production Board and later the War Food Administration, certified weekly the amounts and types of insecticides needed nationwide in crop production areas. As a result, no pesticide-rationing or serious insect outbreaks occurred. The War Production Board was established in 1942 and the War Food Administration in 1943, and both were terminated in 1945.

The forward-looking, progressive leadership fostered by federal entomologists during the mid-1900s has continued in the entomology research community for over five decades. The literature is replete with reports of many outstanding developments of nonchemical insect pest controls. Entomologists also play an important role in U.S. military organizations by supplying medical entomology expertise to support Department of Defense forces worldwide.

Present

Entomological research is a major component of ARS's work. The agency's research has undergone and continues to undergo review and analysis to improve and refocus efforts as needs arise. ARS is committed to excellence, and the research review process is vital to ensuring optimum use of funding and human resources to achieve the best possible environment for creative thinking, program prioritization, and research management strategies.

The entomology program in ARS evolved from the work of a single USDA entomologist in 1854 in Washington, D.C., to now more than 400 entomologists in over 40 locations throughout the United States and various locations abroad. Over the years, the agency has remarkably managed to remain an efficient and productive agricultural research organization in a rapidly developing nation and world with changing needs.

ARS has provided a continuing program of long-term, high-risk, fundamental entomology research that provides a solid base for ensuring continued improvement in agricultural systems technology. Customer and stakeholder satisfaction remains high as shown by the continuing level of program support and receptiveness to ARS prioritization of research and productive contributions. Congressional appropriations for entomological research in 2002 were about $105 million.

Future

The science of entomology has the unique task of understanding some 80 percent of the species on Earth, of which perhaps 85 percent remain uncollected and undescribed. About one-third of the world's crop production depends directly or indirectly on pollination by insects. The overall value of pollination in the world, most of it by insects, has been estimated at about $117 billion per year. The overall value of natural biological control, again mostly by insects, is over $400 billion per year. The value of nutrient cycling in terrestrial ecosystems is over $3 trillion per year. Much of the nutrient cycling is done by insects and related arthropods, which can compose half the animal biomass in some tropical forests. On the other hand, as pests and human competitors, insects destroy or eat produce valued at $5 billion; as disease vectors, they weaken or kill 200 million people per year.

The need to expand entomological and all agricultural research in the future is obvious. Worldwide, current farm values of crop and animal production are estimated at more than $1.3 trillion. Various authors have suggested that farm production losses by insect and mite pests appear to be in the range of 10 to 15 percent with additional losses of 10 to 40 percent occurring during post-harvest handling. Cost of insecticides in the United States in 1995 was $2.1 billion and currently is estimated to equal or exceed $120 billion worldwide. ARS entomologists are committed to reducing or preventing these losses and to controlling costs.

Over the past two decades, world food production in most countries has outpaced population growth. However, a continuation of this trend is not assured. Measurable increases in numbers of undesirable pests moving across regional, national, and international boundaries have occurred with the rapid expansion and improvements of transportation systems. Invasive species cost well over $100 billion annually in the United States. The Invasive Species Council was established in 1999 by Presidential Executive Order 13112. Invasive species were defined as any plant, animal, or organism that is not native to the ecosystem and that is likely to cause harm to human health or the environment or to cause economic losses. In invasive species research, high priority is being placed on reducing the rate of introduction of invasive species; developing detection, identification, and eradication technology for newly introduced pests; and managing and controlling populations of invasive species that have already become established.

The world's human population exceeds 6 billion people. If the population growth rate is 1-2 percent annually, an additional 160-320 thousand people are added to the world population daily. These and similar demographics have intrigued and challenged scientists to develop new food and fiber production technology to provide for the needs of the growing population. Since the 1960s, worldwide agricultural production has increased 80 percent.

The competitive struggles between humans and arthropod pests for the products of mankind's agricultural labors have existed since the beginning of time. The revolutionary discovery of DDT and subsequently thousands of other synthetic organic chemicals for insect control have placed insecticides in the forefront of insect control methodology. Now, the future of the insecticide era has become clouded with issues of heavy reliance, misuse, and insecticide overuse in some instances. Threat to human health, development of insect resistance, environmental contamination, effect on nontarget organisms, and development of secondary pests have been reported. A formidable challenge to research entomologists and other scientists today and in the future is to maintain or increase crop and animal production but provide alternatives to chemical control of pests.

IPM (integrated pest management) continues to be foremost among the advanced concepts to provide economically, environmentally, and socially acceptable insect control. IPM originally addressed insect management but was broadened to include diseases, weeds, and other pests. The origin of the terminology can be traced from integrated control, which became synonymous with IPM and pest management. Successful IPM programs have provided economic benefits to farmers and more environmentally acceptable crop protection practices. Today's exciting success stories from the IPM work of ARS entomologists are surely just a glimpse of what's to come in terms of developing socially, environmentally, and economically sound insect pest control.—By **Thomas J. Henneberry, James R. Coppedge,** and **Larry D. Chandler.**

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